THE
BIOLOGICAL BULLETIN

PUBLISHED BY
THE MARINE BIOLOGICAL LABORATORY

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THE BIOLOGICAL BULLETIN

PUBLISHED BY THE MARINE BIOLOGICAL LABORATORY

THE MARINE BIOLOGICAL LABORATORY

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FORTY-SIXTH YEAR

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II. ACT OF INCORPORATION

No. 3170

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer, and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office;

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips, and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord One Thousand Eight Hundred and Eighty-Eight.

[SEAL]

HENRY B. PIERCE,
Secretary of the Commonwealth.

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 11.30 A.M., daylight saving time, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk to serve one year, and eight Trustees to serve four years. There shall be thirty-two Trustees thus chosen divided into four classes, each to serve four years, and in addition there shall be two groups of Trustees as follows: (*a*) Trustees *ex officio,* who shall be the

President of the Corporation, the Director of the Laboratory, the Associate Director, the Treasurer and the Clerk; (*b*) Trustees Emeritus, who shall be elected from the Trustees by the Corporation. Any regular Trustee who has attained the age of seventy years shall continue to serve as Trustee until the next annual meeting of the Corporation, whereupon his office as regular Trustee shall become vacant and be filled by election by the Corporation and he shall become eligible for election as Trustee Emeritus for life. The Trustees *ex officio* and Emeritus shall have all rights of the Trustees except that Trustees Emeritus shall not have the right to vote.

The Trustees and officers shall hold their respective offices until their successors are chosen and have qualified in their stead.

II. Special meetings of the members may be called by the Trustees to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President of the Corporation who shall also be Chairman of the Board of Trustees; they shall appoint a Director of the Laboratory; and they may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The accounts of the Treasurer shall be audited annually by a certified public accountant.

VIII. The consent of every Trustee shall be necessary to dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be disposed of in such manner and upon such terms as shall be determined by the affirmative vote of two-thirds of the Board of Trustees.

IX. These By-laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. THE REPORT OF THE TREASURER

To the Trustees of the Marine Biological Laboratory:

Gentlemen: Herewith is submitted my report as Treasurer of the Marine Biological Laboratory for the year 1933.

The accounts have been audited by Messrs. Seamans, Stetson, and Tuttle, certified public accountants, and a copy of their report is on file at the Laboratory and is open to inspection by members of the Corporation.

At the end of the year 1933 the book value of the Endowment Funds in the hands of the Central Hanover Bank and Trust Company was as follows:

General Fund,	securities	$ 912,230.91
	in cash	53.08
Library Fund,	securities	196,374.00
	in cash	70.00
Total book value		$1,108,727.99

The income collected from these Funds was as follows:

General Endowment	$40,528.28
Library	$ 8,538.89

Income in arrears, some of which may never be collected, was on December 31:

General Fund	$4,696.66
Library Fund	$1,253.50

The Reserve Fund was partially liquidated at a profit of over $800 and $11,000 applied to the reduction of the Devil's Lane mortgage, leaving a balance of securities and cash in the Fund at the end of the year of the book value of $11,921.17.

The Retirement Fund consisted of securities of the book value of $20,060.00
Cash 3,103.66

Total $23,163.66

Income in arrears on December 31 was $121.61.

The land, buildings, equipment and library, exclusive of the Gansett and Devil's Lane tracts, represented an investment of ... $1,701,816.19
less reserve for depreciation 369,943.83

$1,331,872.36

Expenses, including $42,488.15 depreciation, exceeded income by $24,673.53. There was expended from current funds $18,798.63 in additions to Plant, mostly for books.

At the end of the year the Laboratory owed $14,000 on Bond and Mortgage, and $8,048.74 on open account and had $5,411.85 in cash and bank accounts.

Following is the balance sheet as of December 31, 1933, the condensed statement of income and outgo, also the surplus account, as set out by the accountants:

EXHIBIT A

MARINE BIOLOGICAL LABORATORY BALANCE SHEET, DECEMBER 31, 1933

Assets

Endowment Assets and Equities:			
Securities and Cash in Hands of Central Hanover Bank and Trust Company (New York), Trustee—Schedules I-a and I-b		$1,108,727.99	
Securities and Cash—Minor Funds—Schedule II		10,648.53	$1,119,376.52
Plant Assets:			
Land—Schedule IV	$ 98,103.05		
Buildings—Schedule IV	1,225,286.12		
Equipment—Schedule IV	170,329.50		
Library—Schedule IV	208,097.52	$1,701,816.19	
Less Reserve for Depreciation		369,943.83	
		$1,331,872.36	
Securities and Cash in Reserve Fund		11,921.17	
Cash in Dormitory Building Fund		713.96	$1,344,507.49
Current Assets:			
Cash		$ 5,411.85	
Accounts—Receivable		10,536.58	
Inventories:			
Supply Department	$ 38,513.31		
Biological Bulletin	9,755.26	48,268.57	
Investments:			
Devil's Lane Property	$ 42,345.83		
Gansett Property	5,250.49		
Stock in General Biological Supply House, Inc.	12,700.00		
Retirement Fund Assets	23,163.66	83,459.98	
Prepaid Insurance		3,400.46	
Items in Suspense (Net)		109.56	151,187.00

Liabilities

Endowment Funds:			
Endowment Funds—Schedule III		$1,108,727.99	
Minor Funds—Schedule III		10,648.53	$1,119,376.52
Plant Funds:			
Donations and Gifts—Schedule III		$1,029,372.61	
Other Investments in Plant from Gifts and Current Funds		314,134.88	
		$1,343,507.49	
Note—Payable, Danchakoff Estate		1,000.00	$1,344,507.49
Current Liabilities and Surplus:			
Mortgage, Devil's Lane Property		$ 14,000.00	
Accounts—Payable:			
Current	$ 3,376.24		
Hixon Electric Co., Deferred	3,672.50	7,048.74	
		21,048.74	
Current Surplus—Exhibit C		130,138.26	$ 151,187.00

EXHIBIT B

Marine Biological Laboratory Income and Expense, Year Ended December 31, 1933

	Total		Net	
	Expense	Income	Expense	Income
Income:				
General Endowment Fund		$ 40,528.28		$ 40,528.28
Library Fund		8,538.89		8,538.89
Gifts		400.00		400.00
Instruction	8,055.21	8,910.00		854.79
Research	4,017.04	12,535.50		8,518.46
Evening Lectures	99.41		99.41	
Biological Bulletin and Membership Dues	8,556.28	8,836.44		280.16
Supply Department—Schedule V	38,018.86	36,853.60	1,165.26	
Mess—Schedule VI	21,095.99	22,837.04		1,741.05
Dormitories—Schedule VII	31,093.23	12,531.56	18,561.67	
(Interest and Depreciation charged to above Three Departments. See Schedules V, VI, and VII)	*36,227.30*			36,227.30
Dividends, General Biological Supply House, Inc.		1,120.00		1,120.00
Rents:				
Danchakoff Cottages	294.31	637.50		343.19
Microscopes		284.20		284.20
Garage, Railway, etc.		142.00		142.00
Newman Cottage	124.04	250.00		125.96
Janitor's House	92.03	360.00		267.97

Interest on Bank Balances		29.96		29.96
Sales of Duplicate Library Sets		232.71		232.71
Sundries		26.16		26.16
Maintenance of Plant:				
New Laboratory Expenses ..	14,890.30		14,890.30	
Chemical and Special Apparatus	11,965.38		11,965.38	
Maintenance, Buildings and Grounds	6,764.24		6,764.24	
Library Department Expenses	7,505.98		7,505.98	
Carpenter Department Expenses	621.17		621.17	
Truck Expenses	914.11		914.11	
Sundry Expenses	83.63		83.63	
Workmen's Compensation Insurance	547.85		547.85	
Pumping Station Expenses ...	338.05		338.05	
General Expenses:				
Administration Expenses	14,454.27		14,454.27	
Endowment Fund Trustee ...	968.50		968.50	
Interest on Loans	75.80		75.80	
Bad Debts	620.67		620.67	
Reserve for Depreciation	42,488.15		42,488.15	
Museum Expenses	2,270.17		2,270.17	
	$179,727.37	$155,053.84	$124 334.61	$ 99,661.08
Excess of Expenses over Income carried to Current Surplus—Exhibit C		24,673.53		24,673.53
		$179,727.37		$124,334.61

EXHIBIT C

MARINE BIOLOGICAL LABORATORY, CURRENT SURPLUS ACCOUNT
YEAR ENDED DECEMBER 31, 1933

Balance, January 1, 1933		$122,306.03
Add:		
Reserve for Depreciation charged to Plant Funds		42,488.15
Cash Transferred from Reserve Fund and used to reduce Mortgage on Devil's Lane Land		11,000.00
		$175,794.18
Deduct:		
Payments from Current Funds during Year for Plant Assets as shown in Schedule IV:		
Buildings	$ 562.70	
Equipment	3,100.47	
Library Books, etc.	15,030.46	
	$18,693.63	

Payments on Danchakoff Mortgage from Current Cash		1,000.00	
Excess of Expenses over Income for Year as shown in Exhibit B		24,673.53	
Pensions and Allowances Paid	$2,210.02		
Less Income of Retirement Fund	921.26	1,288.76	45,655.92
Balance, December 31, 1933—Exhibit A			$130,138.26

Respectfully submitted,

LAWRASON RIGGS, JR.,

Treasurer.

V. REPORT OF THE LIBRARIAN

The Library budget until February 6, 1933, was in accordance with that of 1932, namely: total, $24,000; apportioned: current books, $300; serials, $6,000; current serial binding, $1,500; express, $300; supplies, $500; back sets, $6,850; salaries, $8,550. It became necessary in February, 1933, for the Laboratory to revise its budget with a view to greater economy, and $5,000 was thus deducted from the Library budget (February 6). This reduction sum was of necessity taken from the "back sets" apportionment, leaving the amount for the year $1,850. Orders already outstanding at this time were laid out on the budget as follows: current books, $218.14; serials, $5,440.71 (about); current binding, $1,404.02; express, $90.29; supplies, $215.70; back sets, $2,113.75 (about); salaries, $7,150; total, $16,632.61; credit from sale of duplicates, $232.71, or a total of $16,399.90. The budget, reduced in 1933 by one salary ($1,400, as explained in the Report for 1932) and again in February, 1933 ($5,000), to $17,600, gave at the time a balance of $1,200.10. Then in April, when very few of the bills had been received and paid, especially the German bills (the German publishers' time of billing has always been delayed until after the actual issue of their publications), the deflation of the American dollar began. How greatly the fall in the comparative value of American currency affected the Library budget is clearly shown by a comparison of the next figures, the money paid out, with the above figures as estimated: current books, $218.14; serials, $6,922.41; current binding, $1,404.02; express, $90.29; supplies, $215.70; back sets, $3,353.65; salaries, $7,150; total, $19,354.21. Adding to the total budget allowed, $17,600, the credit due to sales, this shows a total deficit of $1,521.50. The addition of the balance that we would have had, had the American dollar remained as it was at the beginning of the year, to the actual deficit as it occurred, gives the total loss to the Laboratory of $2,721.60 due to deflation.

Fortunately for us, the German scientific publications have been reduced in price twenty to thirty per cent over last year's price, and the amount per year is fixed. We hope also that the mark will not increase to a value greater than $.40 during the year. For these reasons, we expect to contine all our current subscriptions in 1934 as we did in 1933. The "back sets" budget must again be sacrificed in so far as is necessary to keep the current serials going. Also, the binding of many volumes of current serials (about 300) selected from the less used must be omitted for the present year.

The Library now contains 37,420 volumes (5,866 books and 31,554 serial volumes), and 81,208 reprints (2,532 bound). Eighty-six new books were purchased and 15 new serials were undertaken in January, 1933, so that the Library now subscribes to 346 current journals (15 "carry-on" book orders were completed and 1 serial dropped); receives by exchange 580 (13 new); and by gift, 211. Four back sets were completed and 8 back sets were partially completed.

For the Woods Hole Oceanographic Institution, there were purchased 18 new books and 3 back sets; 5 exchanges were undertaken. A complete rendering of the expenditure of the $1,000 allowed by the Woods Hole Oceanographic Institution for the year can also be made, although their fiscal year is not closed until February 28. The $1,000 was spent approximately as follows: books, $54.81; serials, $554.21; binding, $43.21; supplies, $3.00; back sets, $344.77. No separate account is to be reported of the total volumes so far purchased for the Woods Hole Oceanographic Institution, as the gain in number is recorded in the total accessions of the Library.

Several notable gifts were made during the year. By Professor Bradley M. Patten, many books and reprints from his father's library were presented. Professor H. McE. Knower gave about 25 books and several hundred reprints; Dr. Robert W. Hegner, several hundred reprints; Dr. E. W. Gudger, several hundred reprints; and Dr. Louis Murbach willed to us his books and pamphlets. From authors, there were received 22 new books, and from publishers as follows: P. Blakiston's Son & Co., 1; Harper & Bros., 4; Lea & Febiger, 3; McGraw-Hill Book Co., Inc., 2; Macmillan Co., 2.

VI. THE REPORT OF THE DIRECTOR

To the Trustees of the Marine Biological Laboratory:

Gentlemen: I beg to submit herewith a report of the forty-sixth session of the Marine Biological Laboratory for the year 1933.

1. *Attendance.* The number of investigators in attendance in 1933 was slightly greater than that in 1932, the figures for the two years being 319 and 314, respectively. At the same time, the registration in the courses of instruction fell from 132 to 118, giving after a correction for duplications a net decrease of 7 in the total attendance. The number of institutions represented by students in the courses was 58 in 1933 as compared with 76 in 1932, but that represented by investigators remained almost unchanged, being 2 less than that of the preceding year and only 10 less than the maximum of 102 reached in 1931. The seasonal distribution of the attendance in 1933, and for comparison, that for the preceding six years, is shown in tabular form.

		1927	1928	1929	1930	1931	1932	1933
May	30	7	15	9	6	6	8	11
June	10	50	64	55	50	51	54	46
"	20	114	140	139	153	153	127	129
"	30	212	240	197	208	217	172	184
July	10	247	281	238	253	258	225	235
"	20	247	282	242	250	273	245	253
"	30	245	272	249	253	281	248	255
August	10	234	250	256	254	302	257	261
"	20	208	226	243	245	280	236	244
"	30	168	183	220	204	239	190	205
September	10	110	112	157	122	136	129	117
"	20	50	43	59	44	69	58	45
"	30	12	14	14	8	14	13	12

2. *The Report of the Treasurer.* As in the case of nearly all educational and scientific institutions, the year 1933 was a particularly unfavorable one for the Laboratory from a financial, though fortunately not from a scientific point of view. The total gross income, which in 1932 had shown a decrease of approximately $29,000.00 from the previous year, declined still further by about $22,000.00 to $155,053.84, which is the lowest figure reached since the establishment of the present endowment in 1925. The loss in gross income was accounted for roughly as follows: income from investments, $6,600.00; subscriptions from institutions, $900.00; sales by the Supply Department, $7,100.00; receipts from the Mess and the dormitories, $4,400.00; miscellaneous, $3,000.00. As to the first of these items, it should be noted that a decrease in income from investments of only approximately 12 per cent

under the conditions prevailing in 1933 indicates an exceptional soundness of the securities in the endowment fund. Few endowed institutions have fared so well. It should be noted further that at least a part of the usual income from the endowment fund which was not received in 1933 will eventually be paid, though to balance this favorable circumstance is the unfavorable one that funds now invested in securities about to mature cannot with equal safety be reinvested so as to yield their present rates of interest. The attention of all the members of the Corporation is invited to the possible opportunities they may have, through the institutions with which they are connected, to prevent further losses in income from the second and third of the sources mentioned above.

In order to carry on its activities within its considerably reduced budget, the Laboratory in 1933 adopted certain economies which, though unwelcome, did not seriously handicap its scientific work. The most important of these was the discontinuance of the use of the steamer "Cayadetta," which to many persons had seemed to be an indispensable part of the equipment of the institution. The experience of the past year, however, has shown that at least the most important scientific needs of the investigators and of the students in the courses can be cared for much less expensively than previously by means of smaller boats, including both those already belonging to the Laboratory and others rented from time to time for special occasions. By means of this major and many minor economies, the expenditures in 1933 were reduced to the point where, except for depreciation charges, the budget was balanced. As has been mentioned in previous reports, the depreciation charges on the expensive buildings and equipment owned by the Laboratory cannot, under present conditions, be met from current income, and an annual deficit in a strict business sense is therefore unavoidable. This deficit for 1933 amounted to $24,673.53.

One of the most favorable points mentioned in the Treasurer's Report is the payment by the Laboratory during 1933 of a considerable part of its indebtedness on the Devil's Lane Tract. Further payments made after the period covered by the report on this and on one other outstanding mortgage have reduced the total amount owed by the Laboratory on mortgages from $27,000.00 in the summer of 1933 to only $2,000.00 in May, 1934. The future savings resulting from the almost complete elimination of interest payments at rates higher than those at present obtainable from first-class securities will be most advantageous to the Laboratory.

3. *The Report of the Librarian.* The Library, handicapped by a reduction of $5,000.00 in its appropriation for the purchase of back sets of journals and by the low purchasing power of the dollar in foreign

countries, has nevertheless maintained, though at a somewhat slower rate, the steady progress it has shown for a number of years past. Its growth since 1925 can most concisely be shown by means of the following table:

	1925	1926	1927	1928	1929	1930	1931	1932	1933
Serials received currently	500	628	764	874	985	1,060	1,080	1,126	1,137
Total number of bound volumes	15,000	18,200	22,800	26,500	28,300	31,500	33,800	36,000	37,400
Reprints ..	25,000	38,000	43,000	51,000	59,000	64,000	70,000	76,000	81,000

Appreciative acknowledgment may be made at this point of the generosity of those mentioned in the Librarian's Report who, during the past year, have presented valuable books and reprints to the Library.

4. *Lectures and Scientific Meetings.* During the summer of 1933, 10 regular evening lectures and also several special lectures were given, and 12 evening scientific sessions were held at which 48 shorter papers on recently completed work were presented by investigators associated with the Marine Biological Laboratory and the Woods Hole Oceanographic Institution. As for several years past, an important feature of the scientific program of the summer was an all-day session held on August 31 at which 31 additional papers, based on work done at the Laboratory during the current season, were presented. Abstracts of most of the shorter scientific contributions will be found in the Biological Bulletin for October, 1933.

5. *Retirement of Employees.* During the past year three persons who have been associated with the Laboratory almost from its beginning retired from active service. Few institutions have been so fortunate in the length and the loyalty of the services of their employees as has the Marine Biological Laboratory in the cases of John J. Veeder, George M. Gray, and Ellis M. Lewis. In recognition of this fact, suitably engrossed letters of appreciation were presented by the Board of Trustees to those retiring, and these letters are here placed on permanent record:

On the Occasion of the Retirement of

CAPTAIN JOHN VEEDER

from active service of the

MARINE BIOLOGICAL LABORATORY

the Trustees offer him their thanks for the faithful perform-

ance of his responsible duties as Captain of the Laboratory Boats for the past

Forty-five Years.

They congratulate him upon his PERFECT RECORD of never having had a serious accident either to life or property, although during these many years he has carried thousands of students and investigators on Collecting Trips and Excursions.

The many friends of "Captain John" unite in wishing for him Health, Happiness and the satisfaction that comes from Work Well Done.

The Trustees of the
Marine Biological Laboratory
hereby express to

GEORGE MILTON GRAY

their satisfaction and pride in his faithful and efficient services, extending over a period of

Forty-two Years

first as Collector, then as Manager of the Supply Department, and finally as Curator of the Museum.

Under his management the Supply Department became an important part of the work of the Laboratory and by his kindness and courtesy Mr. Gray has endeared himself to a host of students and investigators.

On the occasion of his retirement from active service, the Trustees extend to him and to Mrs. Gray their best wishes for continued health and happiness.

"Well done, Good and Faithful Servant."

The Trustees of the
Marine Biological Laboratory
hereby record their appreciation
of the faithful services of

ELLIS M. LEWIS

who for a period of

Thirty-six Years

served the Laboratory as

CHIEF ENGINEER OF THE LABORATORY BOATS.

On the Occasion of his Retirement from Active Duties they extend to him their best wishes for continued usefulness and happiness.

6. *The Board of Trustees.* At the Annual Meeting of the Corporation held on Tuesday, August 8, Dr. William Ruthrauff Amberson of the University of Tennessee and Dr. Carl Caskey Speidel of the University of Virginia were elected Trustees to fill vacancies in the Class of 1937.

There are appended as parts of this Report:

1. The Staff, 1933.
2. Investigators and Students, 1933.
3. A Tabular View of Attendance, 1929–1933.
4. Subscribing and Coöperating Institutions, 1933.
5. Evening Lectures, 1933.
6. Shorter Scientific Papers, 1933.
7. Members of the Corporation. August, 1933.

Respectfully submitted,

M. H. JACOBS,

Director.

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1. THE STAFF, 1933

MERKEL H. JACOBS, *Director*, Professor of General Physiology, University of Pennsylvania.
Associate Director: ——

ZOÖLOGY

I. INVESTIGATION

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.
E. G. CONKLIN, Professor of Zoölogy, Princeton University.
CASWELL GRAVE, Professor of Zoölogy, Washington University.
H. S. JENNINGS, Professor of Zoölogy, Johns Hopkins University.
FRANK R. LILLIE, Professor of Embryology, The University of Chicago.
C. E. MCCLUNG, Professor of Zoölogy, University of Pennsylvania.
S. O. MAST, Professor of Zoölogy, Johns Hopkins University.
T. H. MORGAN, Director of the Biological Laboratory, California Institute of Technology.
G. H. PARKER, Professor of Zoölogy, Harvard University.
E. B. WILSON, Professor of Zoölogy, Columbia University.
LORANDE L. WOODRUFF, Professor of Protozoölogy, Yale University.

II. INSTRUCTION

T. H. BISSONNETTE, Professor of Biology, Trinity College.
E. C. COLE, Professor of Biology, Williams College.
B. R. COONFIELD, Instructor in Biology, Brooklyn College.
C. E. HADLEY, Associate Professor of Biology, New Jersey State Teachers College at Montclair.

O. E. NELSEN, Instructor in Zoölogy, University of Pennsylvania.
S. A. MATTHEWS, Associate in Anatomy, School of Medicine, University of Pennsylvania.
L. P. SAYLES, Instructor in Biology, College of the City of New York.

JUNIOR INSTRUCTORS

F. R. HAYES, Associate Professor of Zoölogy, Dalhousie University.
F. H. WOODS, Assistant Professor of Zoölogy, University of Missouri.

PROTOZOÖLOGY

I. INVESTIGATION

(*See Zoölogy*)

II. INSTRUCTION

GARY N. CALKINS, Professor of Protozoölogy, Columbia University.
RACHEL BOWLING, Instructor in Zoölogy, Columbia University.
ROBERT W. STABLER, Instructor in Zoölogy, University of Pennsylvania.

EMBRYOLOGY

I. INVESTIGATION

(*See Zoölogy*)

II. INSTRUCTION

L. G. BARTH, Instructor of Experimental Zoölogy, Columbia University.
HUBERT B. GOODRICH, Professor of Biology, Wesleyan University.
BENJAMIN H. GRAVE, Professor of Biology, De Pauw University.
LEIGH HOADLEY, Professor of Zoölogy, Harvard University.
CHARLES PACKARD, Assistant Professor of Zoölogy, Institute of Cancer Research, Columbia University.

PHYSIOLOGY

I. INVESTIGATION

HAROLD C. BRADLEY, Professor of Physiological Chemistry, University of Wisconsin.
WALTER E. GARREY, Professor of Physiology, Vanderbilt University Medical School.
RALPH S. LILLIE, Professor of General Physiology, The University of Chicago.
ALBERT P. MATHEWS, Professor of Biochemistry, University of Cincinnati.

II. INSTRUCTION

Teaching Staff

WILLIAM R. AMBERSON, Professor of Physiology, University of Tennessee.
ROBERT CHAMBERS, Prpfessor of Biology, New York University.
RALPH W. GERARD, Associate Professor of Physiology, The University of Chicago.

LAURENCE IRVING, Associate Professor of Physiology, University of Toronto.
BALDUIN LUCKÉ, Associate Professor of Pathology, University of Pennsylvania.
LEONOR MICHAELIS, Member of the Rockefeller Institute, New York City.
MARGARET SUMWALT, Assistant Instructor, University of Pennsylvania Medical School.

BOTANY

I. INVESTIGATION

C. E. ALLEN, Professor of Botany, University of Wisconsin.
S. C. BROOKS, Professor of Zoölogy, University of California.
B. M. DUGGAR, Professor of Physiological and Economic Botany, University of Wisconsin.
IVEY F. LEWIS, Professor of Biology, University of Virginia.
WM. J. ROBBINS, Professor of Botany, University of Missouri.

II. INSTRUCTION

WILLIAM RANDOLPH TAYLOR, Professor of Botany, University of Michigan.
HUGH P. BELL, Professor of Botany, Dalhousie University.
G. W. PRESCOTT, Assistant Professor of Biology, Albion College.

GENERAL OFFICE

F. M. MACNAUGHT, Business Manager.
POLLY L. CROWELL, Assistant.

RESEARCH SERVICE AND GENERAL MAINTENANCE

SAMUEL E. POND, Technical Manager.
OSCAR W. RICHARDS, Chemical Service.
G. FAILLA, X-Ray Physicist.
THOMAS E. LARKIN, Superintendent.
WILLIAM HEMENWAY, Carpenter.
LESTER F. BOSS, Research Technician.
J. D. GRAHAM, Glassblower.
P. H. LILJESTRAND, Assistant.

LIBRARY

PRISCILLA B. MONTGOMERY (Mrs. Thomas H. Montgomery, Jr.), Librarian.
DEBORAH LAWRENCE, Secretary.
DORIS ENDREJAT, MARY A. ROHAN, Assistants.

SUPPLY DEPARTMENT

JAMES MCINNIS, Manager.
JOHN J. VEEDER, Captain.
ELLIS M. LEWIS, Engineer.
A. M. HILTON, Collector.
A. W. LEATHERS, Shipping Department.
MILTON B. GRAY, Collector.
GEOFFREY LEHY, Collector.
WALTER KAHLER, Collector.

MUSEUM

GEORGE M. GRAY, Curator.

2. INVESTIGATORS AND STUDENTS, 1933

Independent Investigators

ADAMS, EDGAR M., Graduate Assistant, University of Cincinnati.
ADAMS, THEODORE G., Instructor in Biology, John Adams High School.
ADDISON, WILLIAM H. F., Professor of Normal Histology and Embryology, University of Pennsylvania.
AMBERSON, WILLIAM R., Professor of Physiology, University of Tennessee.
ANDERSON, RUBERT S., Research Associate, Princeton University.
ARMSTRONG, PHILIP B., Assistant Professor of Anatomy, Cornell University Medical College.
BACQ, Z. M., Assistant in Laboratory of Physiology, University of Liège, Liège, Belgium.
BAILEY, RALPH J., Instructor in Zoölogy. George Washington University.
BAITSELL, GEORGE A., Professor of Biology, Yale University.
BALL, ERIC G., Instructor in Chemistry, Johns Hopkins Medical School.
BARRON, E. S. GUZMAN, Assistant Professor of Biochemistry, The University of Chicago.
BARTH, L. G., Instructor, Columbia University.
BEAMS, H. W., Assistant Professor of Zoölogy, State University of Iowa.
BELL, HUGH P., Professor of Botany, Dalhousie University.
BISSONNETTE, T. HUME, Professor of Biology, Trinity College.
BLINKS, LAWRENCE R., Associate, General Physiology, Rockefeller Institute.
BODANSKY, AARON, Biochemist, Research Laboratory, Hospital for Joint Diseases, New York City, N. Y.
BODINE, J. H., Professor and Head of Zoölogy, State University of Iowa.
BOWEN, RUFUS E., Assistant Professor of Biology, Long Island University.
BOWLING, RACHEL, Instructor in Zoölogy, Columbia University.
BRADLEY, H. C., Professor of Physiological Chemistry, University of Wisconsin.
BRINLEY, F. J., Assistant Professor of Zoölogy and Physiology, North Dakota State College.
BROOKS, M. M., Research Associate in Biology, University of California.
BROOKS, S. C., Professor of Zoölogy, University of California.
BROWN, DUGOLD E. S., Assistant Professor of Physiology, New York University and Bellevue Medical College.
BUDINGTON, R. A., Professor of Zoölogy, Oberlin College.
BULMER, GLADYS, Teacher of Biology, University of Pennsylvania.
BUTLER, ELMER G., Associate Professor of Biology, Princeton University.
CALKINS, GARY N., Professor of Protozoölogy, Columbia University.
CANNAN, ROBERT K., Professor of Chemistry, University and Bellevue Hospital Medical College.
CARPENTER, RUSSELL L., Associate in Anatomy, College of Physicians and Surgeons, Columbia University.
CATTELL, WARE, Garrison-on-Hudson, New York.
CHAMBERS, ROBERT, Research Professor of Biology, Washington Square College, New York University.
CHENEY, RALPH H., Chairman, Biology Department, Long Island University.
CHESLEY, LEON C., Assistant Biophysicist, Memorial Hospital.
CHIDESTER, F. E., Professor of Zoölogy, West Virginia University.
CLARK, ELEANOR L., University of Pennsylvania.
CLARK, ELIOT R., Professor of Anatomy, University of Pennsylvania.
CLOWES, GEORGE H. A., Director of Research, Lilly Research Laboratories.
COE, W. R., Professor of Biology, Yale University.
COGHILL, GEORGE E., Member, Wistar Institute of Anatomy and Biology.
COLE, ELBERT C., Professor of Biology, Williams College.

COLLIP, J. B., Professor of Biochemistry, McGill University.
CONKLIN, E. G., Professor of Biology, Princeton University.
COONFIELD, BENJAMIN R., Instructor in Biology, Brooklyn College.
COPELAND, MANTON, Professor of Biology, Bowdoin College.
COWLES, RHEINART P., Professor of Zoölogy, Johns Hopkins University.
CROASDALE, HANNAH T., Graduate Student, University of Pennsylvania.
DAWSON, ALDEN B., Associate Professor of Zoölogy, Harvard University.
DAY, DOROTHY, Assistant Professor of Botany, Smith College.
DILLER, WILLIAM F., Instructor in Zoölogy, Dartmouth College.
DONALDSON, HENRY H., Member, Wistar Institute of Anatomy and Biology.
DONALDSON, JOHN C., Professor of Anatomy, University of Pittsburgh, Medical School.
EDWARDS, DAYTON J., Associate Professor of Physiology, Cornell University Medical College.
EDWARDS, THOMAS I., Instructor in Biology, School of Hygiene, Johns Hopkins University.
ENDERS, ROBERT K., Assistant Professor of Zoölogy, Swarthmore College.
FINLEY, HAROLD E., Associate Professor of Biology, West Virginia State College.
FLEISHER, MOYER S., Professor of Bacteriology and Hygiene, St. Louis University.
FOWLER, VIRGINIA M., Assistant in Botany, Barnard College.
FRY, HENRY J., Professor of Biology, Washington Square College, New York University.
FURTH, JACOB, Assistant Professor in Pathology, Cornell University Medical College.
FURTOS, NORMA C., Fellow in Biology, Western Reserve University.
GARREY, W. E., Professor of Physiology, Vanderbilt University, School of Medicine.
GATES, GORDON E., Head of Department of Biology, Judson College.
GEIMAN, QUENTIN M., Research Assistant, University of Pennsylvania.
GERARD, R. W., Associate Professor of Physiology, The University of Chicago.
GILMORE, KATHRYN A., Instructor in Botany, Pennsylvania College for Women.
GOLDFORB, A. J., Professor of Biology, College of the City of New York.
GOODRICH, H. B., Professor of Biology, Wesleyan University.
GRAUBARD, MARC A., Department of Zoölogy, Columbia University.
GRAVE, B. H., Professor of Zoölogy, DePauw University.
GRAVE, CASWELL, Professor of Zoölogy, Washington University.
HADLEY, CHARLES E., Associate Professor of Biology, State Teachers College at Montclair.
HALL, EDMUND K., Instructor, University of Louisville, School of Medicine.
HAMBURGER, VICTOR, Assistant, University of Frieburg, Frieburg, Germany.
HARNLY, MORRIS H., Assistant Professor, Washington Square College, New York University.
HARTLINE, H. K., Lecturer in Medical Physics, University of Pennsylvania.
HARVEY, ETHEL BROWNE, Research Worker, Princeton University.
HARVEY, E. NEWTON, Professor of Physiology, Princeton University.
HAYES, FREDERICK R., Associate Professor of Zoölogy, Dalhousie University.
HAYWOOD, CHARLOTTE, Associate Professor of Physiology, Mount Holyoke College.
HEILBRUNN, L. V., Associate Professor of Zoölogy, University of Pennsylvania.
HENSHAW, P. S., Biophysicist, Memorial Hospital, New York City.
HETHERINGTON, W. ALFORD, Bruce Fellow, Johns Hopkins University.
HIBBARD, HOPE, Associate Professor, Oberlin College.
HILL, SAMUEL E., Assistant in General Physiology, Rockefeller Institute.
HOADLEY, LEIGH, Professor of Zoölogy, Harvard University.
HOLLAENDER, ALEXANDER, National Research Fellow in Biological Science, University of Wisconsin.

HOPPE, ELLA N., Bacteriologist, New York State Department of Health.
HOWARD, EVELYN, Instructor in Physiology, Johns Hopkins Medical School.
HOWE, H. E., Editor, Industrial and Engineering Chemistry.
HUSSEY, KATHLEEN L., Assistant in Zoölogy, Connecticut College.
HUTNER, SEYMOUR H., Graduate Student, Cornell University.
HYDE, IDA H., Emeritus Professor of Physiology, State University of Kansas.
IRVING, LAURENCE, Associate Professor of Physiology, University of Toronto.
JACOBS, M. H., Professor of General Physiology, University of Pennsylvania.
JASPER, HERBERT H., Research Fellow, National Research Council and Brown University.
JENKINS, GEORGE B., Professor and Director Department of Anatomy, George Washington University.
JENNINGS, H. S., Professor of Zoölogy, Johns Hopkins University.
JOHLIN, J. M., Associate Professor of Biochemistry, Vanderbilt Medical School.
KEIL, ELSA MARIE, Instructor in Zoölogy, New Jersey College for Women.
KELLEY, TRUMAN L., Professor in Graduate School of Education, Harvard University.
KEYES, DONALD B., Professor of Chemical Engineering, University of Illinois.
KIDDER, GEORGE W., Tutor, College of the City of New York.
KIRKPATRICK, T. BRUCE, Associate Professor of Physical Education, Columbia University.
KNOWLTON, FRANK P., Professor of Physiology, Syracuse University, College of Medicine.
KORR, IRVIN M., Assistant Instructor, Princeton University.
KROGH, MARIE, University of Copenhagen, Denmark.
LANCEFIELD, D. E., Associate Professor of Zoölogy, Columbia University.
LANCEFIELD, REBECCA C., Associate in Bacteriology, Rockefeller Institute for Medical Research.
LAUG, EDWIN P., Instructor in Physiology, University of Pennsylvania.
LILLIE, FRANK R., Chairman of the Department of Zoölogy, The University of Chicago.
LILLIE, MARGARET CRANE, Boston City Hospital.
LILLIE, RALPH S., Professor of General Physiology, The University of Chicago.
LUCKÉ, BALDUIN, Professor of Pathology, University of Pennsylvania.
LYNCH, RUTH STOCKING, Instructor, Johns Hopkins University.
MACDOUGALL, MARY STUART, Professor of Zoölogy, Agnes Scott College.
MCGREGOR, J. H., Professor of Zoölogy, Columbia University.
MACHLIS, SAMUEL, Graduate Assistant in Biology, Washington Square College, New York University.
MAGRUDER, SAMUEL R., Graduate Assistant in Zoölogy, University of Cincinnati.
MARTIN, EARL A., Assistant Professor, Chairman of Biology Department, Brooklyn College.
MAST, S. O., Professor of Zoölogy, Johns Hopkins University.
MATHEWS, A. P., Carnegie Professor of Biochemistry, University of Cincinnati.
MATTHEWS, SAMUEL A., Associate in Anatomy, University of Pennsylvania.
MENKIN, VALY, Instructor in Pathology, Harvard University Medical School.
MICHAELIS, DR. LEONOR, Member, Rockefeller Institute for Medical Research.
MILLER, FORREST W., Graduate Assistant, University of Pittsburgh.
MOMENT, GAIRDNER B., Instructor in Biology, Goucher College.
MORELAND, FERRIN B., Assistant in Biochemistry, Vanderbilt Medical School.
MORGAN, LILIAN V., California Institute of Technology.
MORGAN, T. H., Professor of Biology, California Institute of Technology.
MORGULIS, SERGIUS, Professor of Biochemistry, University of Nebraska, College of Medicine.
MORRILL, E. V., Associate Professor of Anatomy, Cornell University Medical College.

NELSEN, OLIN E., Instructor in Zoölogy, University of Pennsylvania.
NONIDEZ, JOSÉ F., Assistant Professor of Anatomy, Cornell University Medical College.
NORTHROP, JOHN H., Member, Rockefeller Institute.
OLTMANN, CLARA E., Tutor, Brooklyn College.
ORR, PAUL R., Instructor, Brooklyn College.
PACKARD, CHARLES, Assistant Professor of Zoölogy, Columbia University.
PALMER, A. H., Teaching Fellow, New York University and Bellevue Medical College.
PALMER, ELIZABETH T., Instructor, Vassar College.
PAPENFUSS, GEORGE F., Instructor in Botany, Johns Hopkins University.
PARKER, G. H., Professor of Zoölogy, Harvard University.
PARPART, ARTHUR K., Assistant Professor of Physiology, Princeton University.
PELLUET, DIXIE, Assistant Professor of Zoölogy, Dalhousie University.
PLOUGH, HAROLD H., Professor of Biology, Amherst College.
POLLISTER, ARTHUR W., Instructor in Zoölogy, Columbia University.
POLLISTER, PRISCILLA FREW, Instructor in Biology, Brooklyn College.
POND, SAMUEL E., Technical Manager, Marine Biological Laboratory.
PRESCOTT, G. W., Assistant Professor of Biology, Albion College.
DE RENYI, GEORGE S., Associate Professor of Anatomy, University of Pennsylvania.
REX, RICHARD O., Instructor in Anatomy, University of Pennsylvania.
REZNIKOFF, PAUL, Assistant Professor of Medicine, Cornell University Medical College.
RICHARDS, OSCAR W., Instructor in Biology, Yale University.
ROOT, WALTER S., Associate Professor of Physiology, College of Medicine, Syracuse University.
RUGH, ROBERTS, Instructor in Zoölogy, Hunter College.
SAYLES, LEONARD P., Instructor in Biology, College of the City of New York.
SCHECHTER, VICTOR, Columbia University.
SCHMIDT, IDA T. GENTHER, Instructor in Histology, Children's Hospital, Cincinnati.
SCHMIDT, L. H., Fellow, Christ Hospital, Cincinnati.
SCHMIEDER, RUDOLF G., Instructor in Zoölogy, University of Pennsylvania.
SCHOTTE, OSCAR E., Sterling Research Fellow, Yale University.
SCHRADER, FRANZ, Professor of Zoölogy, Columbia University.
SCHRADER, SALLY HUGHES, Professor of Zoölogy, Sarah Lawrence College.
SCOTT, ALLAN C., Assistant in Zoölogy, Columbia University.
SCOTT, SISTER FLORENCE MARIE, Graduate Student, Columbia University.
SHOUP, CHARLES S., Assistant Professor of Biology, Vanderbilt University.
SHUMWAY, WALDO, Professor of Zoölogy, University of Illinois.
SICHEL, FERDINAND J. M., Assistant Instructor, Washington Square College, New York University.
SLIFER, ELEANOR H., Research Associate in Zoölogy, State University of Iowa.
SMITH, DIETRICH C., Harvard University.
SMITH, PHILIP E., Professor of Anatomy, Columbia University.
SNELL, GEORGE D., National Research Fellow, University of Texas.
SONNEBORN, TRACY M., Research Associate, Johns Hopkins University.
SPEICHER, B. R., Graduate Assistant, University of Pittsburgh.
SPEIDEL, CARL C., Professor of Anatomy, University of Virginia Medical School.
SPEK, JOSEF, Professor of Zoölogy, University of Heidelberg, Germany.
STABLER, ROBERT M., Instructor in Zoölogy, University of Pennsylvania.
STEINBACH, H. BURR, National Research Fellow, The University of Chicago.
STEWART, DOROTHY R., Assistant Professor of Biology, Skidmore College.
STOCKARD, CHARLES R., Professor of Anatomy, Cornell University Medical College.
STOKEY, ALMA G., Professor of Botany, Mount Holyoke College.

STUNKARD, HORACE W., Professor of Biology, New York University.
STURTEVANT, A. H., Professor of Genetics, California Institute of Technology.
SUMWALT, MARGARET, Assistant Instructor, University of Pennsylvania Medical School.
SWEADNER, WALTER R., Graduate Assistant, University of Pittsburgh.
TASHIRO, DR. SHIRO, Professor of Biochemistry, University of Cincinnati, College of Medicine.
TAYLOR, G. WELLFORD, National Research Fellow, Princeton University.
TAYLOR, WILLIAM R., Professor of Botany, University of Michigan.
TENNENT, DAVID H., Professor of Biology, Bryn Mawr College.
TULLOCH, GEORGE S., Instructor, Brooklyn College.
VAN CLEAVE, HARLEY J., Professor of Zoölogy, University of Illinois.
VICARI, EMILIA M., Associate in Anatomy, Cornell University Medical College.
WATERMAN, ALLYN J., Instructor in Biology, Brooklyn College.
WEICHERT, CHARLES K., Assistant Professor of Zoölogy, University of Cincinnati.
WEISMAN, MAXWELL N., Tutor, College of the City of New York.
WHEDON, ARTHUR D., Professor and Head of Zoölogy and Physiology, North Dakota Agricultural College.
WHITING, ANNA R., Professor and Head of Department of Biology, Pennsylvania College for Women.
WHITING, P. W., Professor of Zoölogy, University of Pittsburgh.
WIEMAN, H. L., Professor of Zoölogy, University of Cincinnati.
WILLEY, CHARLES H., Assistant Professor of Biology, University College, New York University.
WILSON, E. B., Da Costa Professor Emeritus in Residence, Columbia University.
WINSOR, CHARLES P., Student, Harvard University.
WOLF, E. ALFRED, Associate Professor of Zoölogy, University of Pittsburgh.
WOODS, FARRIS H., Assistant Professor of Zoölogy, University of Missouri.
WOODWARD, ALVALYN E., Assistant Professor, University of Michigan.
YOUNG, ROGER ARLINER, Assistant Professor of Zoölogy, Howard University.
YOUNG, WILLIAM C., Assistant Professor of Biology, Brown University.
ZIRKLE, CONWAY, Associate Professor, University of Pennsylvania.
ZIRKLE, RAYMOND E., National Research Council Fellow, Johnson Foundation, University of Pennsylvania.

Beginning Investigators

ABRAMOWITZ, ALEXANDER A., Harvard University.
AGATE, FREDERIC J., Graduate Student, Columbia University.
ALBAUM, HARRY G., Fellow, Brooklyn College.
ATLAS, MEYER, Assistant in Zoölogy, Columbia University.
BADEN, VICTOR, Graduate Student and University Scholar, University of Pennsylvania.
BECK, LYLE V., Graduate Assistant in Zoölogy, University of Pittsburgh.
BOSTIAN, CAREY H., Assistant Professor of Zoölogy, North Carolina State College.
BRIDGES, JOHN C., Instructor in Zoölogy, Morehouse College.
CARLSON, J. GORDON, Instructor in Biology, Bryn Mawr College.
CARLETON, BLONDEL H., Fellow in Physiology, University of Rochester Medical School.
CHAO, IPING, Graduate Student, The University of Chicago.
CHEN, TZE-TUAN, Instructor, University of Pennsylvania.
CLARK, JEAN M., Graduate Student, University of Pennsylvania.
COHEN, ROSE S., Graduate Assistant in Zoölogy, University of Cincinnati.
COLE, MARGARET GRIERSON, Mount Holyoke College.
COSTELLO, DONALD P., Instructor in Zoölogy, University of Pennsylvania.
CRAMPTON, C. B., Instructor in Biology, Wesleyan University.
DAN, KATSUMA, Graduate Student, University of Pennsylvania.

DERRICKSON, MARY B., Frederica, Delaware.
DRUMTRA, ELIZABETH, Assistant in Zoölogy, Barnard College, Columbia University.
DUNCAN, PAUL M., Graduate Student, University of Pennsylvania.
EASTLICK, HERBERT L., Graduate Assistant in Zoölogy, Washington University.
FARROW, JOHN G., High School Instructor and Student, University of Pennsylvania.
HAVEY, CLINTON B., Acadia University.
HEGNAUER, ALBERT H., Assistant in Physiology, University of Rochester.
HICKS, FREDERICK JAMES, Graduate Student in Zoölogy, University of Pittsburgh.
HIRSCHFELD, NATHAN B., Graduate Student, Columbia University.
HUNTER, LAURA, Graduate Student, University of Pennsylvania.
JOHNSON, ARLENE C., Graduate Assistant, Oberlin College.
KAGAN, BENJAMIN M., Washington and Jefferson College.
KALISS, NATHAN, Student, Columbia University.
KEKWICK, RALPH A., Visiting Fellow, Princeton University.
KELTCH, ANNA K., Research Assistant, Lilly Research Laboratories.
KYLE, JOHN ARTHUR, Explorers Club, New York.
LANDOWNE, MILTON, Student, Harvard Medical School.
LAWLOR, SISTER ANNA CATHERINE, Instructor in Biology, College of Saint Elizabeth.
LIEDKE, KATHE B., Student, Columbia University.
MARSHAK, ALFRED, Student, Harvard University.
MARSLAND, DOUGLAS A., Assistant Professor of Biology, Washington Square College, New York University.
MAXWELL, JANE, Instructor in Biology, Carnegie Institute of Technology.
MAZIA, DANIEL, Graduate in Zoölogy, University of Pennsylvania.
MELAMPY, ROBERT M., Assistant in Animal Nutrition, Cornell University.
MENKIN, MIRIAM F., Research Fellow in Pathology, Harvard Medical School.
METZNER, JEROME, Graduate Student, Columbia University.
MILLER, JAMES A., Graduate Assistant in Zoölogy, The University of Chicago.
MORRIS, SAMUEL, Instructor, Temple University.
MOSER, FLOYD, Graduate Student, University of Pennsylvania.
NICHOL, MARGARET A., University of Pittsburgh.
NOVIKOFF, ALEX BENJAMIN, Fellow, Brooklyn College.
RANKIN, JOHN S., Assistant, Wesleyan University.
RUBENSTEIN, BORIS B., Assistant in Physiology, The University of Chicago.
RUBIDGE, KARYL W., Student, Vassar College.
RUSSELL, W. LAWSON, Fellow, The University of Chicago.
SAUER, FRED C., Assistant Professor, University of Kansas.
SCHWEITZER, MORTON D., Assistant in Zoölogy, Columbia University.
SHAPIRO, HERBERT, Graduate Student, Princeton University.
SPECHT, HEINZ, Graduate Student, Johns Hopkins University.
STONE, FAITH, Scholar, The University of Chicago.
STRICKER, GEORGE J., Student, Yale University.
STUART, MARTHA S., University of Pittsburgh.
SUMMERS, FRANCIS M., Student, Columbia University.
TOWNSEND, GRACE, Assistant, The University of Chicago.
WALKER, PAUL A., Graduate Assistant, Harvard University.
WEBSTER, M. DOROTHY, Dalhousie University.
WICHTERMAN, RALPH, Graduate Student, University of Pennsylvania.
WILSON, HILDEGARD, Assistant, New York University.

Research Assistants

ANTHONY, GENEVIEVE, University of Pennsylvania.
ARMSTRONG, LOUISE S., Research Assistant, Cornell University Medical College.

AUERBUCK, SYLVIA, Student, College of the City of New York.
BARON, HARRY, Assistant Instructor, New York University.
BLACK, PETER, Student, McGill University.
BROWN, WILLIAM R., Graduate Student, University of Cincinnati.
BUCK, LOUISE H., 113 South Grove Street, East Orange, New Jersey.
BUTT, CHARLES, Research Assistant, Princeton University.
COGHILL, MURIEL, Fallsington, Pennsylvania.
CORSON, SAMUEL A., Research Assistant, University of Pennsylvania.
DENNY, MARTHA, Radcliffe College.
DOYLE, WILLIAM L., Brooks Fund Fellow, Johns Hopkins University.
ENGEL, FRANK L., Student, Dartmouth College.
ENGEL, GEORGE L., Undergraduate, Dartmouth College.
FISHER, KENNETH C., Fellow in Department of Physiology, University of Toronto.
FRANCIS, DOROTHY S., Research Assistant, Memorial Hospital, New York City.
GAMMON, GEORGE D., Johnson Foundation, University of Pennsylvania.
GRAND, C. G., Research Assistant, New York University.
HAMBURGER, MARTHA, Chicago, Illinois.
HARNLY, MARIE L., Assistant in Biology, New York University.
HILL, EDGAR S., Research Assistant, Rockefeller Institute.
HOIJER, DOROTHY J., Assistant, The University of Chicago.
JAO, CHIN-CHIH, Assistant, University of Michigan.
KATZ, JACOB D., Research Assistant, St. Andrews University, Scotland.
LANCEFIELD, STUART, Student, Columbia University.
LARRABEE, MARTIN G., 4402 Pine Street, Philadelphia, Pennsylvania.
LUNDSTROM, HELEN M., 511 Kenwood Road, Drexel Hill, Pennsylvania.
MCCROAN, J. E., Graduate Assistant, State University of Iowa.
MCLANE, KATHRYN, Teacher of Biology, Annapolis High School.
MANERY, JEANNE F., Fellow in Physiology, University of Toronto.
PORTER, HELEN, Laboratory Assistant, Harvard University.
RICHARDSON, MARGARET S., Cornell University Medical College.
ROBERTSON, LOLA ELLIS, Research Assistant, New York University.
RUSCH, ELIZABETH, Research Assistant, Memorial Hospital.
SELL, JAMES P., Graduate Assistant, Yale University.
SHAW, ISIDOR, Research Assistant, Long Island University.
SMITH, CHARLES E., III, Medical Student, University of Pennsylvania.
STARKEY, WILLIAM F., Graduate Assistant, University of Pittsburgh.
STEIN, MARTIN H., Student, Cornell University Medical College.
STUART, RICHARD R., Graduate Assistant, State University of Iowa.
WADE, LUCILLE, Research Assistant, Lilly Research Laboratories.
WALLACE, EDITH M., Scientific Artist, Carnegie Institution of Washington.
ZUJKO, ALPHONSE J., Research Assistant, Trinity College.

Students

BOTANY

AMIDON, ELAINE WILSON, Syracuse University.
BOSWORTH, MILLARD W., Assistant in Biology, Wesleyan University.
CAMPBELL, MILDRED F., 29 N. Hawthorne La., Indianapolis, Indiana.
CUNNIFF, HILDA S., 1140 E. Market Street, Indianapolis, Indiana.
PORIS, ETHEL, Hunter College.
RAMEY, SALLY, Student, Elmira College.

EMBRYOLOGY

ALBAUM, HARRY G., Fellow, Brooklyn College.
ARMACK, CLIFFORD M., Curator of Biology, Museum of Northern Arizona.
BATES, M. NOBLE, Graduate Assistant in Zoölogy, Oberlin College.

BELL, RUTH, Wellesley College.
BENGEL, WALTER Z., Assistant in Anatomy and Embryology, DePauw University.
CHEN, I., Graduate Student, University of Pennsylvania.
CHURNEY, LEON, Graduate Student, University of Pennsylvania.
DEWOLF, ROBERT A., Instructor in Zoölogy, Rhode Island State College.
FOSTER, EDITH F., Undergraduate Student, Vassar College.
GODWIN, MELVIN C., Assistant in Histology and Embryology, Cornell University.
CRECO, FILOMENA M., Hunter College.
HAMILTON, MARY ALICE, Elmira College.
HOOPER, KATHRYN T., Wheaton College.
KAGAN, BENJAMIN M., Washington and Jefferson College.
KRIETE, FREDERIC M., Student, DePauw University.
LIPPMAN, RICHARD W., Yale University.
MCAULEY, AULEY A., Student, DePauw University.
MCGEHEE, ELISE, Newcomb College, New Orleans.
MOSER, FLOYD, Graduate Student, University of Pennsylvania.
ROOT, CHARLOTTE M., Ohio Wesleyan University.
ROSE, SYLVAN M., Graduate Assistant, Amherst College.
RUBIDGE, KARYL W., Student, Vassar College.
SPANGLER, ELIZABETH A., Wheaton College.
TAYLOR, HOYT C., Graduate Assistant, Wesleyan University.
TUKEY, GERTRUDE R., Undergraduate, Smith College.
TURNER, ROBERT S., Instructor, Dartmouth College.
WARDWELL, JUDITH S., Laboratory Assistant, Wellesley College.
ZINN, DONALD J., Student, Harvard University.

PHYSIOLOGY

ALT, HOWARD L., Associate in Medicine, Northwestern University Medical School.
BOTSFORD, E. FRANCES, Assistant Professor of Zoölogy, Connecticut College.
DERRICKSON, MARY B., Frederica, Delaware.
GLASSMEYER, ELMER J., Graduate Student, University of Cincinnati.
HAVEY, CLINTON B., Acadia University.
HIBBARD, JEANNE, Oberlin College.
HOWELL, CHARLES D., Johns Hopkins University.
HULL, FRANK M., Head of Department of Physiology, University of Mississippi.
JOHNSON, EDNA L., Associate Professor of Biology, University of Colorado.
MCINTOSH, FRANKLIN C., Demonstrator in Pharmacology, Dalhousie University.
MATHEWS, ROBERT S., 49 West 52d Street, New York City, New York.
MELAMPY, ROBERT M., Assistant in Animal Nutrition, Cornell University.
MORELAND, FERRIN B., Graduate Assistant, Vanderbilt Medical School.
ROSS, EDWARD, Student, University of California.
SOLANDT, DONALD Y., Research Fellow, University of Toronto.
SOLANDT, OMOND MCK., Research Assistant, University of Toronto.
STRICKER, GEORGE J., Undergraduate, Yale University.
VEXLER, DARWIN E., Student, Rutgers University.
WEBSTER, MARGARET D., Dalhousie University.

PROTOZOÖLOGY

BECHTEL, WILMER R., Columbia University.
BOYER, DADE C., Douglas School.
DENNIS, NOVA N., 1595 Woodward Avenue, Lakewood, Ohio.
HIRSCHFELD, NATHAN B., Columbia University.
MOORE, RAYMOND P., West Virginia State College.
ORBISON, AGNES M., Professor of Biology, Elmira College.
PERKINS, IRENE T., Teacher of Biology, Columbia University.

STUBBS, TRAWICK H., Instructor in Biology, Emory Junior College.
URBAN, JOHN, Columbia University.
WARD, MARY, Wellesley College.
YOUNG, MARTIN D., Instructor in Biology, Junior College of Augusta.

INVERTEBRATE ZOÖLOGY

ABRAMOWITZ, ALEXANDER A., Graduate Student, Harvard University.
BARTHOLOMEW, OLIVE F., Radcliffe College.
BLADES, HELEN N., Teacher, University of Michigan.
BLAGG, AMY ELIZABETH, Assistant Professor of Zoölogy, Grinnell College.
BOSWORTH, MILLARD W., Assistant in Biology, Wesleyan University.
BOWMAN, SARAH B., Assistant, Agnes Scott College.
BROOKS, VIRGINIA C., Student, Wilson College.
BUCK, JOHN B., Assistant in Zoölogy, Johns Hopkins University.
BUELL, KATHERINE M., Oberlin College.
CARMACK, TED, Student Assistant, Wabash College.
CLARK, FRANCES J., Student, University of Rochester.
COLLINGS, WILLIAM D., Undergraduate Laboratory Assistant, DePauw University.
COWLES, JANET M., Johns Hopkins University.
DENNY, MARTHA, Student, Radcliffe College.
DiPAOLA, ROSE M., Hunter College.
FIELD, MARY F., Laboratory Assistant, Brearley School.
GAW, HARRY, Student, Yale University.
GIDDINGS, W. PHILIP, Student, Amherst College.
HENDERSON, ALLEN R., Student, Yale University.
HORTON, RICHARD G., Student, Williams College.
HUNTER, FRANCIS R., California Institute of Technology.
JONES, LEO M., Undergraduate Laboratory Assistant, DePauw University.
JONES, ROY W., Associate Professor of Biology, Oklahoma University.
KELLY, FLORENCE C., Instructor, Simmons College.
LAGLER, KARL F., University of Rochester.
LIVINGSTON, MARY C., Student Assistant, American University.
MAST, LOUISE R., Graduate Student, Johns Hopkins University.
METCALF, ISAAC S. H., Undergraduate, Oberlin College.
MILLER, THOMAS R., Undergraduate, Hamilton College.
NICHOLS, RAY J., Graduate Student, University of Illinois.
ODELL, FLOYD A., Assistant in Zoölogy, Yale University.
PAINTER, BEN T., Instructor in Zoölogy, College of William and Mary.
PARKER, RACHEL W., Goucher College.
PODOLSKY, SOPHIA, Goucher College.
ROGERS, PHILIP V., Instructor, Hamilton College.
ROHM, PAULINE B., Oberlin College.
SHAW, RUTH K., Student, Mount Holyoke College.
SHEPARD, MARGARET J., Graduate Assistant, Mt. Holyoke College.
SMITH, STUART D., Student, Wabash College.
SPIEGEL, JOHN P., Dartmouth College.
STARRETT, WILLIAM C., University of Illinois.
STEWART, JOHN T., JR., 300 Court Street, Portsmouth, Virginia.
STONE, FAITH, Student, The University of Chicago.
STUART, MARTHA S., Pennsylvania College for Women.
TAYLOR, HOYT C., Graduate Assistant in Biology, Wesleyan University.
TODD, ROBERT E., JR., Harvard University.
TREZISE, WILLARD J., Assistant in Zoölogy, Johns Hopkins University.
VAN DEVENTER, WILLIAM C., Graduate Assistant, University of Illinois.
WHARTON, MARGUERITE, Student Assistant, New Jersey State Teachers College.
WING, LUCIUS T., Undergraduate, Harvard University.

WHITTINGHILL, MAURICE, Instructor, Dartmouth College.
WILLIAMS, INEZ W., Massachusetts State College.
WILLIAMS, MARGARITA, University of Iowa.
WOODSIDE, GILBERT, Assistant in Zoölogy, Harvard University.

3. TABULAR VIEW OF ATTENDANCE

	1929	1930	1931	1932	1933
INVESTIGATORS—Total	329	337	362	314	319
Independent	234	217	236	212	210
Under Instruction	71	87	83	73	66
Research Assistants	24	33	43	29	43
STUDENTS—Total	125	136	125	132	118
Zoölogy	53	56	55	55	54
Protozoölogy	15	14	17	16	11
Embryology	28	27	29	29	28
Physiology	17	23	17	18	19
Botany	12	16	7	14	6
TOTAL ATTENDANCE	454	473	487	446	437
Less Persons registered as both students and investigators	10	14	20	14	12
	444	459	467	432	425
INSTITUTIONS REPRESENTED—Total	123	126	137	141	120
By Investigators	96	95	102	94	92
By Students	64	71	68	76	58
SCHOOLS AND ACADEMIES REPRESENTED					
By Investigators	—	—	—	—	1
By Students	1	4	4	1	2
FOREIGN INSTITUTIONS REPRESENTED					
By Investigators	30	7	8	8	5
By Students	3	2	1	1	—

4. SUBSCRIBING AND COÖPERATING INSTITUTIONS, 1933

American University
Amherst College
Atlanta University
Bowdoin College
Bryn Mawr College
Chinese Educational Mission
College of St. Elizabeth
Columbia University
Commonwealth Fund
Cornell University
Cornell University Medical College
C. R. B. Foundation
De Pauw University
Duke University
General Education Board
Goucher College
Hamilton College
Harvard University
Harvard University Medical School
Hunter College
Industrial & Engineering Chemistry, of the American Chemical Society
Johns Hopkins University

Johns Hopkins University Medical School
Eli Lilly & Co.
Long Island University
Memorial Hospital of N. Y. City
Morehouse College
Mount Holyoke College
Museum of Northern Arizona
New York State Department of Health
New York University
New York University Medical School
Oberlin College
Pennsylvania College for Women
Princeton University
Radcliffe College
Rockefeller Foundation
Rockefeller Institute for Medical Research
Rutgers University
Smith College
Sophie Newcomb College
Swarthmore College
Syracuse University
Tufts College
University of Chicago
University of Cincinnati
University of Iowa
University of Pennsylvania
University of Pennsylvania Medical School
University of Pittsburgh
University of Rochester
University of Rochester Medical School
University of Wisconsin
Vanderbilt University Medical School
Vassar College
Wabash College
Wellesley College
Wesleyan University
West Virginia State College
Wheaton College
Williams College
Wilson College
Wistar Institute of Anatomy and Biology
Yale University

5. EVENING LECTURES, 1933

Friday, July 7
Dr. G. H. Parker ..... "Transmission of Neurohumoral Substances."

Tuesday, July 11
Dr. The Svedberg ..... "Ultracentrifugal and Cataphoretic Studies on Respiratory Proteins."

Friday, July 21
Dr. Balduin Lucké ..... "The Zoölogical Distribution of Tumors."

Friday, July 28
Dr. Laurence Irving ..... "On the Ability of Mammals to Survive Without Breathing."

Wednesday, August 2
Dr. Oscar E. Schotté ..... "Organizers and Inherent Potencies in the Embryonic Development of Amphibians."

Friday, August 4
Dr. August Krogh ..... "Conditions of Life in the Depths of the Ocean."

Friday, August 11
Dr. F. Spek ..... "Die Protoplasmadifferenzierung der Eizellen während der ersten Entwicklung."

Friday, August 18
Dr. John H. Northrop ..... "Evidence of the Protein Nature of Pepsin and Trypsin."

Friday, August 25
Dr. Edwin Grant Conklin ..... "Science and Progress."

Friday, September 1
Dr. Robert Keith Cannan ..... "Studies in the Amphoteric Properties of Proteins."

SPECIAL LECTURES AND MOTION PICTURES

Saturday, July 1
Dr. A. V. Hill ..... "Nerve."

Monday, July 17
Dr. Robert Chambers ..... Motion pictures of studies on the insemination and segmentation of marine ova.

Thursday, August 24
Dr. Robert Chambers and
Mr. C. G. Grand ..... "Tissue Culture Technique and Various Aspects of the Growth of Normal and Cancerous Tissues."

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6. SHORTER SCIENTIFIC PAPERS, 1933

Friday, June 30
Dr. Ethel B. Harvey ..... "Effects of Centrifugal Force on the Ectoplasmic Layer, Nuclei, and Protoplasm of Fertilized Sea Urchin Eggs."
Dr. E. Newton Harvey ..... Motion pictures taken through the centrifuge-microscope illustrating differences in behavior of unfertilized and fertilized sea urchin eggs.
Dr. William C. Young ..... "Some Data from a Correlated Anatomical, Physiological, and Behavioristic Study of the Reproductive Cycle in the Female Guinea Pig."
Dr. William R. Amberson ..... "Hæmoglobin-Ringer, a New Mammalian Perfusion Fluid."

Wednesday, July 5
Dr. G. W. Prescott ..... "Some Effects of Blue-green Algæ on Lake Fish."
Dr. Hugh P. Bell ..... "Distribution and Ecology of the Marine Algæ of the Maritime Provinces of Canada."

Dr. W. R. Taylor ..... "Distribution of Newfoundland Algæ."

Monday, July 10

Dr. A. C. Redfield ..... "The Concentration of Organic Derivatives in Sea Water, in Relation to the Chemical Composition of Plankton."

Dr. George L. Clarke ..... "Diurnal Migration of Plankton in the Gulf of Maine and Its Correlation with Changes in Submarine Irradiation."

Dr. Selman A. Waksman and
Miss Cornelia L. Carey ..... "The Rôle of Bacteria in the Formation of Nitrate in the Sea."

Friday, July 14

Dr. M. M. Brooks ..... "The Effect of Respiratory Poisons and Methylene Blue on Cleavage of Certain Eggs."

Dr. Laurence Irving and
Miss Jeanne F. Manery ..... "Ionic Changes During the Development of Fish Eggs."

Dr. E. Newton Harvey ..... "The Tension at the Surface of Egg Cells."

Mr. Donald P. Costello ..... "Fertilization Membranes of Centrifuged Asterias Eggs."

Dr. Robert Chambers ..... "The Kidney Tubules with Phenol Red and Neutral Red."

Tuesday, July 18

Dr. G. W. Kidder ..... "Chromatin Extrusion in Certain Ciliate Commensals of Mussels."

Mr. F. M. Summers ..... "The Reorganization Bands in the Macronucleus of Aspidisca."

Mr. Harold E. Finley ..... "Comparative Studies on the Osmiophilic and Neutral-Red-Stainable Inclusions of the Genus Vorticella."

Mr. William L. Doyle ..... "Experimental Cytology of Amœba proteus."

Tuesday, July 25

Dr. H. B. Goodrich and
Mr. C. B. Crampton ..... "One Step in the Development of Hereditary Pigmentation in the Fish Oryzias latipes."

Dr. George D. Snell ..... "Translocations in the Mouse and Their Effect on Development."

Dr. D. E. Lancefield ..... "A Series of Probable Mutations in Drosophila pseudo-obscura as Compared with D. melanogaster."

Dr. P. W. Whiting ..... "Sex-determination in Hymenoptera."

Tuesday, August 1

Dr. Conway Zirkle ..... "The Effects of Fat Solvents Upon the Fixation of Mitochondria."

Dr. Arthur W. Pollister ..... "The Cytology of Amphibian Tissues."

Dr. B. M. Duggar and
Dr. Alexander Hollaender .... "The Irradiation of Biological Suspensions by Monochromatic Light. (The effect of ultra-violet light on a plant virus and bacteria.)"

Dr. C. C. Speidel ..... Motion pictures showing some varieties of nerve irritation, as seen in living frog tadpoles.

Tuesday, August 8

Dr. Edmund K. Hall ..... "Regional Differences in the Organization Center of the Amphibian Embryo."

Dr. George A. Baitsell ..... "Observations on Migrating Cells in Cultures of Amphibian Tissues, Particularly with Reference to the Problem of Fiber Formation."

Dr. V. Hamburger ..... "The Effect of Wing Bud Extirpations in Chick Embryos on the Development of the Nervous System."

Dr. G. H. Parker ..... "Color Changes in the Dogfish."

Thursday, August 10

Dr. A. B. Dawson ..... "The Absorption of Colloidal Carbon by the Mesonephric Epithelium of Necturus."

Mr. Victor Schechter ..... "Morphological and Electrophoretic Effects of the Galvanic Current on Griffithsia Cells."

Mr. K. Dan ..... "The Electric Charge on the Surface of Sea Urchin Eggs."

Dr. R. W. Gerard ..... "Electrical Activity of the Brain."

Tuesday, August 15

Dr. A. H. Palmer ..... "The Isolation of a Crystalline Globulin from the Albumin Fraction of Cow's Milk."

Dr. Paul Reznikoff ..... "Studies in Iron Metabolism in Humans."

Dr. Marie Krogh ..... "The Hormonal Connection between the Pituitary and the Thyroid."

Dr. F. E. Chidester ..... "Anterior Pituitary Like Hormone Effects."

Tuesday, August 22

Mr. R. Rugh ..... "Heterochromatic Radiation and Early Amphibian Development."

Dr. R. E. Zirkle ..... "A Non-linear Relation Between Biological Effect and Ionizing Power of Alpha Rays."

Dr. Leon C. Chesley ..... "Effects of X-rays Upon Cell Oxidations."

Dr. P. S. Henshaw and
Dr. D. S. Francis ..... "A Response of Arbacia Eggs to X-rays."

Tuesday, August 29

Dr. Charles B. Wilson ..... "The Copepod Plankton of the Last Cruise of the Non-magnetic Ship 'Carnegie.'"

Dr. Dugald E. S. Brown ..... "The Pressure Coefficient of Viscosity in the Eggs of Arbacia."

Dr. Edwin P. Laug ..... "Observations on Lactic Acid, Total CO_2, and pH of Venous Blood During Recovery from Severe Exercise."

Dr. Anna R. Whiting ..... "A Study of Eye Color in the Parasitic Wasp, Habrobracon."

Dr. Marc A. Graubard ..... "The Melanin Reaction in Races of Drosophila melanogaster."

Thursday, August 31

Dr. Paul A. Reznikoff and
Mrs. Dorothy G. Reznikoff .... "Blood Cell Studies in Dogfish."

Dr. W. H. F. Addison ..... "Intracranial Pigmentation in Teleosts."

Dr. E. R. Clark and
Mrs. Eleanor Linton Clark ... "The Blood Capillary in Relation to Contractility."

Mr. Herbert L. Eastlick ..... "Striated Muscles of the Lamellibranch Mollusc, Pecten gibbus."

Dr. Arthur W. Pollister ..... "The Centrioles of Amphibian Tissues."

Mr. Theodore G. Adams ..... "The Chromidium in Arcella vulgaris."

Dr. Harold H. Plough ..... "Selective Fertilization in Styela."

Mr. Meyer Atlas ..... "Relation of Temperature and Cleavage in Frogs' Eggs."

Dr. E. G. Conklin ..... "Disorientations of Development in Crepidula, Caused by Cold."

Dr. Ethel Browne Harvey ..... "Changes in the Arbacia Egg Immediately Following Fertilization, as Determined by Centrifugal Force."

Dr. P. S. Henshaw and
Dr. D. S. Francis ..... "Recovery from X-ray Effects Before Fertilization in Arbacia Eggs and Its Effect on Development."

Miss Anna K. Keltch,
Miss Lucille Wade and
Dr. G. H. A. Clowes ....."Further Observations on the Contrasting Sensitivity of Eggs and Sperm to Various Chemical Agents."

Dr. G. H. A. Clowes,
Miss Anna K. Keltch and
Miss Lucille Wade ....."Variations in the Sensitivity of Eggs Following Fertilization."

Dr. E. Newton Harvey ....."Flattening of Marine Eggs Under the Influence of Gravity."

Dr. L. V. Heilbrunn ....."The Action of Anæsthetics on the Surface Precipitation Reaction."

Dr. Dorothy R. Stewart and
Dr. M. H. Jacobs ....."The Effect of Certain Salt Solutions on the Permeability of the Arbacia Egg."

Dr. B. R. Speicher ....."The Effective Period in Development of the Mutant Factor 'Eyeless' in Habrobracon."

Dr. Anna R. Whiting ....."Variegated Eye Color in Habrobracon."

Dr. P. W. Whiting ....."Egg-trinuclearity in Habrobracon."

Mr. Heinz Specht ....."Relation Between Oxygen Tension and Respiration in Spirostomum ambiguum, with Corrections for Ammonia."

Dr. C. S. Shoup ....."Respiration and Luminescence of Bacteria in Carbon Monoxide."

Dr. G. Wellford Taylor ....."The Relation Between Luminescence and Respiration in Bacteria with Especial Reference to the Effects of Narcotics."

Dr. Lyle V. Beck ....."Nature of the Aerobic Apparent Reduction Potential."

Dr. Eric G. Ball ....."The Relative Abundance of Hydrogen Isotopes in Sea Water."

Dr. Oscar W. Richards ....."Toxicity of Some Metals and Berkefeld Filtered Sea Water to Mytilus Edulis."

Dr. Herbert H. Jasper ....."Some New Aspects of the Physiology of the Nerve-Muscle System in Crustacea Brought Out by Electrical Excitation and Response."

Dr. Margaret Sumwalt and
Miss Kathryn McLane ....."The Blood Pressure of Limulus."

Mr. John C. Bridges and
Dr. Margaret Sumwalt ....."The Effect of pH Upon Potassium Penetration into Fundulus Eggs."

DR. ARTHUR K. PARPART ....."A Method for Following Volume Changes of Cells."

DR. WILLIAM R. AMBERSON,
MR. FRANK ENGEL,
MISS DOROTHY WEBSTER AND
DR. EDWIN P. LAUG ....."The Influence of pH upon the Passage of Hæmoglobin Through the Glomerulus of the Perfused Frog's Kidney."

DR. M. H. JACOBS AND
DR. ARTHUR K. PARPART ....."The Influence of the Escape of Salts on the Osmotic Behavior of the Erythrocyte."

---

7. MEMBERS OF THE CORPORATION

1. LIFE MEMBERS

ALLIS, MR. E. P., JR., Palais Carnoles, Menton, France.
ANDREWS, MRS. GWENDOLEN FOULKE, Baltimore, Md.
BILLINGS, MR. R. C., 66 Franklin St., Boston, Mass.
CONKLIN, PROF. EDWIN G., Princeton University, Princeton, N. J.
COOLIDGE, MR. C. A., Ames Building, Boston, Mass.
CRANE, MR. C. R., New York City.
EVANS, MRS. GLENDOWER, 12 Otis Place, Boston, Mass.
FAY, MISS S. B., 88 Mt. Vernon St., Boston, Mass.
FOOT, MISS KATHERINE, Care of Morgan Harjes Cie, Paris, France.
GARDINER, MRS. E. G., Woods Hole, Mass.
JACKSON, MISS M. C., 88 Marlboro St., Boston, Mass.
JACKSON, MR. CHAS. C., 24 Congress St., Boston, Mass.
KIDDER, MR. NATHANIEL T., Milton, Mass.
KING, MR. CHAS. A.
LEE, MRS. FREDERIC S., 279 Madison Ave., New York City, N. Y.
LOWELL, MR. A. LAWRENCE, 17 Quincy St., Cambridge, Mass.
MCMURRICH, PROF. J. P., University of Toronto, Toronto, Canada.
MEANS, DR. JAMES HOWARD, 15 Chestnut St., Boston, Mass.
MERRIMAN, MRS. DANIEL, 73 Bay State Road, Boston, Mass.
MINNS, MISS SUSAN, 14 Louisburg Square, Boston, Mass.
MORGAN, MR. J. PIERPONT, JR., Wall and Broad Sts., New York City, N. Y.
MORGAN, PROF. T. H., Director of Biological Laboratory, California Institute of Technology, Pasadena, Calif.
MORGAN, MRS. T. H., Pasadena, Calif.
NOYES, MISS EVA J.

OSBORN, PROF. HENRY F., American Museum of Natural History, New York, N. Y.
PHILLIPS, MRS. JOHN C., Windy Knob, Wenham, Mass.
PORTER, DR. H. C., University of Pennsylvania, Philadelphia, Pa.
SEARS, DR. HENRY F., 86 Beacon St., Boston, Mass.
SHEDD, MR. E. A.
THORNDIKE, DR. EDWARD L., Teachers College, Columbia University, New York City, N. Y.
TRELEASE, PROF. WILLIAM, University of Illinois, Urbana, Ill.
WARE, MISS MARY L., 41 Brimmer St., Boston, Mass.
WILSON, DR. E. B., Columbia University, New York City, N. Y.

2. REGULAR MEMBERS, 1933

ADAMS, DR. A. ELIZABETH, Mount Holyoke College, South Hadley, Mass.
ADDISON, DR. W. H. F., University of Pennsylvania Medical School, Philadelphia, Pa.
ADOLPH, DR. EDWARD F., University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.
ALLEE, DR. W. C., University of Chicago, Chicago, Ill.
ALLYN, DR. HARRIET M., Mount Holyoke College, South Hadley, Mass.
AMBERSON, DR. WILLIAM R., University of Tennessee, Memphis, Tenn.
ANDERSON, DR. E. G., California Institute of Technology, Pasadena, Calif.
ARMSTRONG, DR. PHILIP B., Cornell University Medical College, 1300 York Avenue, New York City, N. Y.
AUSTIN, DR. MARY L., Wellesley College, Wellesley, Mass.
BAITSELL, DR. GEORGE A., Yale University, New Haven, Conn.
BALDWIN, DR. F. M., University of Southern California, Los Angeles, Calif.
BALL, DR. ERIC G., Johns Hopkins Medical School, Baltimore, Md.
BARD, PROF. PHILIP, Johns Hopkins Medical School, Baltimore, Md.
BARRON, DR. E. S. GUZMAN, Department of Medicine, The University of Chicago, Chicago, Ill.
BARTH, DR. L. G., Columbia University, New York City, N. Y.
BECKWITH, DR. CORA J., Vassar College, Poughkeepsie, N. Y.
BEHRE, DR. ELINOR H., Louisiana State University, Baton Rouge, La.
BENNITT, DR. RUDOLF, University of Missouri, Columbia, Mo.
BIGELOW, DR. H. B., Museum of Comparative Zoölogy, Cambridge, Mass.
BIGELOW, PROF. R. P., Massachusetts Institute of Technology, Cambridge, Mass.

BINFORD, PROF. RAYMOND, Guilford College, Guilford College, N. C.
BISSONNETTE, DR. T. H., Trinity College, Hartford, Conn.
BLANCHARD, PROF. KENNETH C., New York University, Washington Square College, New York City, N. Y.
BODINE, DR. J. H., University of Iowa, Iowa City, Ia.
BORING, DR. ALICE M., Yenching University, Peking, China.
BOWLING, MISS RACHEL, Columbia University, New York City, N. Y.
BOX, MISS CORA M., University of Cincinnati, Cincinnati, O.
BRADLEY, PROF. HAROLD C., University of Wisconsin, Madison, Wis.
BRAILEY, MISS MIRIAM E., 710 North Broadway, Baltimore, Md.
BRIDGES, DR. CALVIN B., California Institute of Technology, Pasadena, Calif.
BRONK, DR. D. W., University of Pennsylvania, Philadelphia, Pa.
BROOKS, DR. S. C., University of California, Berkeley, Calif.
BUCKINGHAM, MISS EDITH N., Sudbury, Mass.
BUDINGTON, PROF. R. A., Oberlin College, Oberlin, O.
BULLINGTON, DR. W. E., Randolph-Macon College, Ashland, Va.
BUMPUS, PROF. H. C., 76 Carlton Road, Waban, Mass.
BYRNES, DR. ESTHER F., 1803 North Camac Street, Philadelphia, Pa.
CALKINS, PROF. GARY N., Columbia University, New York City, N. Y.
CALVERT, PROF. PHILIP P., University of Pennsylvania, Philadelphia, Pa.
CANNAN, PROF. R. K., University and Bellevue Hospital Medical College, New York City, N. Y.
CARLSON, PROF. A. J., University of Chicago, Chicago, Ill.
CAROTHERS, DR. E. ELEANOR, University of Pennsylvania, Philadelphia, Pa.
CARPENTER, DR. RUSSELL L., College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York City, N. Y.
CARROLL, PROF. MITCHEL, Franklin and Marshall College, Lancaster, Pa.
CARVER, PROF. GAIL L., Mercer University, Macon, Ga.
CATTELL, DR. MCKEEN, Cornell University Medical College, 1300 York Avenue, New York City, N. Y.
CATTELL, PROF. J. MCKEEN, Garrison-on-Hudson, N. Y.
CATTELL, MR. WARE, Garrison-on-Hudson, N. Y.
CHAMBERS, DR. ROBERT, Washington Square College, New York University, Washington Square, New York City, N. Y.
CHARLTON, DR. HARRY H., University of Missouri, Columbia, Mo.
CHENEY, DR. RALPH H., Biology Department, Long Island University, Brooklyn, N. Y.
CHIDESTER, PROF. F. E., West Virginia University, Morgantown, W. Va.

CHILD, PROF. C. M., The University of Chicago, Chicago, Ill.
CLAPP, PROF. CORNELIA M., Montague, Mass.
CLARK, PROF. E. R., University of Pennsylvania, Philadelphia, Pa.
CLELAND, PROF. RALPH E., Goucher College, Baltimore, Md.
CLOWES, PROF. G. H. A., Eli Lilly & Co., Indianapolis, Ind.
COE, PROF. W. R., Yale University, New Haven, Conn.
COHN, DR. EDWIN J., 183 Brattle Street, Cambridge, Mass.
COLE, DR. ELBERT C., Williams College, Williamstown, Mass.
COLE, DR. KENNETH S., College of Physicians and Surgeons, 630 West 168th Street, New York City, N. Y.
COLE, DR. LEON J., College of Agriculture, Madison, Wis.
COLLETT, DR. MARY E., Western Reserve University, Cleveland, O.
COLTON, PROF. H. S., Box 127, Flagstaff, Ariz.
CONNOLLY, DR. C. J., Catholic University, Washington, D. C.
COONFIELD, DR. B. R., Brooklyn College, 80 Willoughby Street, Brooklyn, N. Y.
COPELAND, PROF. MANTON, Bowdoin College, Brunswick, Me.
COWDRY, DR. E. V., Washington University, St. Louis, Mo.
CRAMPTON, PROF. H. E., Barnard College, Columbia University, New York City, N. Y.
CRANE, MRS. C. R., Woods Hole, Mass.
CURTIS, DR. MAYNIE R., Crocker Laboratory, Columbia University, New York City, N. Y.
CURTIS, PROF. W. C., University of Missouri, Columbia, Mo.
DAVIS, DR. ALICE R., Castle Point, Hoboken, N. J.
DAVIS, DR. DONALD W., College of William and Mary, Williamsburg, Va.
DAWSON, DR. A. B., Harvard University, Cambridge, Mass.
DAWSON, DR. J. A., The College of the City of New York, New York City, N. Y.
DEDERER, DR. PAULINE H., Connecticut College, New London, Conn.
DODDS, PROF. G. S., Medical School, University of West Virginia, Morgantown, W. Va.
DOLLEY, PROF. WILLIAM L., University of Buffalo, Buffalo, N. Y.
DONALDSON, PROF. H. H., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.
DONALDSON, DR. JOHN C., University of Pittsburgh, School of Medicine, Pittsburgh, Pa.
DREW, PROF. GILMAN A., Eagle Lake, Fla.
DUBOIS, DR. EUGENE F., Cornell University Medical College, 1300 York Avenue, New York City, N. Y.
DUGGAR, DR. BENJAMIN M., University of Wisconsin, Madison, Wis.

DUNGAY, DR. NEIL S., Carleton College, Northfield, Minn.
DUNN, DR. L. C., Columbia University, New York City, N. Y.
EDWARDS, DR. D. J., Cornell University Medical College, 1300 York Avenue, New York City, N. Y.
ELLIS, DR. F. W., Monson, Mass.
FARNUM, DR. LOUISE W., Hsiang-Ya Hospital, Changsha, Hunan, China.
FAURÉ-FREMIET, PROF. EMMANUEL, Collège de France, Paris, France.
FENN, DR. W. O., Rochester University, School of Medicine, Rochester, N. Y.
FLEISHER, DR. MOYER S., St. Louis University, School of Medicine, St. Louis, Mo.
FORBES, DR. ALEXANDER, Harvard University Medical School, Boston, Mass.
FRY, DR. HENRY J., Cornell University Medical College, 1300 York Avenue, New York City, N. Y.
GAGE, PROF. S. H., Cornell University, Ithaca, N. Y.
GARREY, PROF. W. E., Vanderbilt University Medical School, Nashville, Tenn.
GATES, PROF. R. RUGGLES, University of London, London, England.
GEISER, DR. S. W., Southern Methodist University, Dallas, Tex.
GERARD, PROF. R. W., The University of Chicago, Chicago, Ill.
GLASER, PROF. O. C., Amherst College, Amherst, Mass.
GOLDFORB, PROF. A. J., College of the City of New York, New York City, N. Y.
GOODRICH, PROF. H. B., Wesleyan University, Middletown, Conn.
GRAHAM, DR. J. Y., University of Alabama, University, Ala.
GRAVE, PROF. B. H., DePauw University, Greencastle, Ind.
GRAVE, PROF. CASWELL, Washington University, St. Louis, Mo.
GRAY, PROF. IRVING E., Duke University, Durham, N. C.
GREENMAN, PROF. M. J., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.
GREGORY, DR. LOUISE H., Barnard College, Columbia University, New York City, N. Y.
GUTHRIE, DR. MARY J., University of Missouri, Columbia, Mo.
GUYER, PROF. M. F., University of Wisconsin, Madison, Wis.
HADLEY, CHARLES E., Teachers' College, Montclair, N. J.
HAGUE, DR. FLORENCE, Sweet Briar College, Sweet Briar, Va.
HALL, PROF. FRANK G., Duke University, Durham, N. C.
HANCE, DR. ROBERT T., University of Pittsburgh, Pittsburgh, Pa.
HARGITT, PROF. GEORGE T., Duke University, Durham, N. C.
HARMAN, DR. MARY T., Kansas State Agricultural College, Manhattan, Kans.

HARNLY, DR. MORRIS H., New York University, Washington Square College, New York City, N. Y.

HARPER, PROF. R. A., Columbia University, New York City, N. Y.

HARRISON, PROF. Ross G., Yale University, New Haven, Conn.

HARVEY, MRS. E. N., Princeton, N. J.

HARVEY, PROF. E. N., Princeton University, Princeton, N. J.

HAYDEN, DR. MARGARET A., Wellesley College, Wellesley, Mass.

HAYWOOD, DR. CHARLOTTE, Mount Holyoke College, South Hadley, Mass.

HAZEN, DR. T. E., Barnard College, Columbia University, New York City, N. Y.

HECHT, DR. SELIG, Columbia University, New York City, N. Y.

HEILBRUNN, DR. L. V., University of Pennsylvania, Philadelphia, Pa.

HENSHAW, DR. PAUL S., Memorial Hospital, 2 West 106th Street, New York City, N. Y.

HESS, PROF. WALTER N., Hamilton College, Clinton, N. Y.

HINRICHS, DR. MARIE A., 1824 Blue Island Avenue, Chicago, Ill.

HISAW, DR. F. L., University of Wisconsin, Madison, Wis.

HOADLEY, DR. LEIGH, Harvard University, Cambridge, Mass.

HOGUE, DR. MARY J., 503 N. High Street, West Chester, Pa.

HOOKER, PROF. DAVENPORT, University of Pittsburgh, Pittsburgh, Pa.

HOPKINS, DR. HOYT S., New York University, College of Dentistry, New York City, N. Y.

HOWE, DR. H. E., 2702 36th Street, N.W., Washington, D. C.

HOWLAND, DR. RUTH B., Washington Square College, New York University, Washington Square, East, New York City, N. Y.

HOYT, DR. WILLIAM D., Washington and Lee University, Lexington, Va.

HUMPHREY, MR. R. R., University of Buffalo, School of Medicine, Buffalo, N. Y.

HYMAN, DR. LIBBIE H., 41 West 70th Street, New York City, N. Y.

IRVING, PROF. LAURENCE, University of Toronto, Toronto, Canada.

JACKSON, PROF. C. M., University of Minnesota, Minneapolis, Minn.

JACOBS, PROF. MERKEL H., University of Pennsylvania, Philadelphia, Pa.

JENKINS, DR. GEORGE B., George Washington University, 1335 M Street, N. W., Washington, D. C.

JENNINGS, PROF. H. S., Johns Hopkins University, Baltimore, Md.

JEWETT, PROF. J. R., Harvard University, Cambridge, Mass.

JOHLIN, DR. J. M., Vanderbilt University Medical School, Nashville, Tenn.

JOHNSON, PROF. GEORGE E., State Agricultural College, Manhattan, Kans.

JORDAN, PROF. E. O., The University of Chicago, Chicago, Ill.
JUST, PROF. E. E., Howard University, Washington, D. C.
KAUFMANN, PROF. B. P., University of Alabama, University, Ala.
KEEFE, REV. ANSELM M., St. Norbert College, West Depere, Wis.
KINDRED, DR. J. E., University of Virginia, Charlottesville, Va.
KING, DR. HELEN D., Wistar Institute of Anatomy and Biology, Philadelphia, Pa.
KING, DR. ROBERT L., State University of Iowa, Iowa City, Ia.
KINGSBURY, PROF. B. F., Cornell University, Ithaca, N. Y.
KNAPKE, REV. BEDE, St. Bernard's College, St. Bernard, Ala.
KNOWER, PROF. H. McE., Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.
KNOWLTON, PROF. F. P., Syracuse University, Syracuse, N. Y.
KOSTIR, DR. W. J., Ohio State University, Columbus, O.
KRIBS, DR. HERBERT, 202A Copley Road, Upper Darby, Pa.
LANCEFIELD, DR. D. E., Columbia University, New York City, N. Y.
LANGE, DR. MATHILDE M., Wheaton College, Norton, Mass.
LEE, PROF. F. S., College of Physicians and Surgeons, New York City, N. Y.
LEWIS, PROF. I. F., University of Virginia, Charlottesville, Va.
LEWIS, PROF. W. H., Johns Hopkins University, Baltimore, Md.
LILLIE, PROF. FRANK R., The University of Chicago, Chicago, Ill.
LILLIE, PROF. RALPH S., The University of Chicago, Chicago, Ill.
LINTON, PROF. EDWIN, University of Pennsylvania, Philadelphia, Pa.
LOEB, PROF. LEO, Washington University Medical School, St. Louis, Mo.
LOWTHER, MRS. FLORENCE DEL., Barnard College, Columbia University, New York City, N. Y.
LUCKÉ, PROF. BALDUIN, University of Pennsylvania, Philadelphia, Pa.
LUSCOMBE, MR. W. O., Woods Hole, M
LYNCH, DR. CLARA J., Rockefeller Institute, New York City, N. Y.
LYNCH, DR. RUTH STOCKING, Johns Hopkins University, Baltimore, Md.
MACDOUGALL, DR. MARY S., Agnes Scott College, Decatur, Ga.
MCCLUNG, PROF. C. E., University of Pennsylvania, Philadelphia, Pa.
MCGREGOR, DR. J. H., Columbia University, New York City, N. Y.
MACKLIN, DR. CHARLES C., School of Medicine, University of Western Ontario, London, Canada.
MALONE, PROF. E. F., University of Cincinnati, Cincinnati, O.
MANWELL, DR. REGINALD D., Syracuse University, Syracuse, N. Y.
MARTIN, PROF. E. A., Cornell University Medical College, 1300 York Avenue, New York City, N. Y.

MAST, PROF. S. O., Johns Hopkins University, Baltimore, Md.
MATHEWS, PROF. A. P., University of Cincinnati, Cincinnati, O.
MATTHEWS, DR. SAMUEL A., Department of Anatomy, University of Pennsylvania, Philadelphia, Pa.
MAVOR, PROF. JAMES W., Union College, Schenectady, N. Y.
MEDES, DR. GRACE, University of Minnesota, Minneapolis, Minn.
MEIGS, DR. E. B., Dairy Division Experiment Station, Beltsville, Md.
MEIGS, MRS. E. B., 1736 M Street, N.W., Washington, D. C.
METCALF, PROF. M. M., 94 Nehoiden Road, Waban, Mass.
METZ, PROF. CHARLES W., Johns Hopkins University, Baltimore, Md.
MICHAELIS, DR. LEONOR, Rockefeller Institute, New York City, N. Y.
MILLER, DR. HELEN M., Washington University, St. Louis, Mo.
MITCHELL, DR. PHILIP H., Brown University, Providence, R. I.
MOORE, DR. CARL R., The University of Chicago, Chicago, Ill.
MOORE, PROF. GEORGE T., Missouri Botanical Garden, St. Louis, Mo.
MOORE, PROF. J. PERCY, University of Pennsylvania, Philadelphia, Pa.
MORGULIS, DR. SERGIUS, University of Nebraska, Lincoln, Nebr.
MORRILL, PROF. A. D., Hamilton College, Clinton, N. Y.
MORRILL, PROF. C. V., Cornell University Medical College, New York City, N. Y.
MULLER, DR. H. J., University of Texas, Austin, Tex.
NABOURS, DR. R. K., Kansas State Agricultural College, Manhattan, Kans.
NEAL, PROF. H. V., Tufts College, Tufts College, Mass.
NEWMAN, PROF. H. H., The University of Chicago, Chicago, Ill.
NICHOLS, DR. M. LOUISE, Rosemont, Pa.
NOBLE, DR. GLADWYN K., American Museum of Natural History, New York City, N. Y.
NONIDEZ, DR. JOSÉ F., Cornell University Medical College, 1300 York Avenue, New York City, N. Y.
OKKELBERG, DR. PETER, University of Michigan, Ann Arbor, Mich.
OSBURN, PROF. R. C., Ohio State University, Columbus, O.
OSTERHOUT, MRS. W. J. V., Rockefeller Institute, New York City, N. Y.
OSTERHOUT, PROF. W. J. V., Rockefeller Institute, New York City, N. Y.
PACKARD, DR. CHARLES, Columbia University, Institute of Cancer Research, 1145 Amsterdam Ave., New York City, N. Y.
PAGE, DR. IRVINE H., Rockefeller Institute, New York City, N. Y.
PAPANICOLAOU, DR. GEORGE N., Cornell University Medical College, 1300 York Avenue, New York City, N. Y.
PAPPENHEIMER, DR. A. M., Columbia University, New York City, N. Y.

PARKER, PROF. G. H., Harvard University, Cambridge, Mass.
PATON, PROF. STEWART, Princeton University, Princeton, N. J.
PATTEN, DR. BRADLEY M., Western Reserve University, Cleveland, O.
PAYNE, PROF. F., University of Indiana, Bloomington, Ind.
PEARL, PROF. RAYMOND, Institute for Biological Research, 1901 East Madison Street, Baltimore, Md.
PEEBLES, PROF. FLORENCE, California Christian College, Los Angeles, Calif.
PHILLIPS, DR. E. F., Cornell University, Ithaca, N. Y.
PINNEY, DR. MARY E., Milwaukee-Downer College, Milwaukee, Wis.
PLOUGH, PROF. HAROLD H., Amherst College, Amherst, Mass.
POLLISTER, DR. A. W., Columbia University, New York City, N. Y.
POND, DR. SAMUEL E., Marine Biological Laboratory, Woods Hole, Mass.
PRATT, DR. FREDERICK H., Boston University, School of Medicine, Boston, Mass.
RAFFEL, DR. DANIEL, Zoölogical Laboratory, Johns Hopkins University, Baltimore, Md.
RAND, DR. HERBERT W., Harvard University, Cambridge, Mass.
REDFIELD, DR. ALFRED C., Harvard University, Cambridge, Mass.
REESE, PROF. ALBERT M., West Virginia University, Morgantown, W. Va.
DE RENYI, DR. GEORGE S., Department of Anatomy, University of Pennsylvania, Philadelphia, Pa.
REZNIKOFF, DR. PAUL, Cornell University Medical College, 1300 York Avenue, New York City, N. Y.
RHODES, PROF. ROBERT C., Emory University, Atlanta, Ga.
RICE, PROF. EDWARD L., Ohio Wesleyan University, Delaware, O.
RICHARDS, PROF. A., University of Oklahoma, Norman, Okla.
RICHARDS, DR. O. W., Osborn Zoölogical Laboratory, Yale University, New Haven, Conn.
RIGGS, MR. LAWRASON, JR., 25 Broad Street, New York City, N. Y.
ROGERS, PROF. CHARLES G., Oberlin College, Oberlin, O.
ROMER, DR. ALFRED S., The University of Chicago, Chicago, Ill.
ROOT, DR. W. S., Syracuse Medical School, Syracuse, N. Y.
SAYLES, DR. LEONARD P., Department of Biology, College of the City of New York, 139th Street and Convent Avenue, New York City, N. Y.
SCHRADER, DR. FRANZ, Department of Zoölogy, Columbia University, New York City, N. Y.
SCHRADER, DR. SALLY HUGHES, Department of Zoölogy, Columbia University, New York City, N. Y.

SCHRAMM, PROF. J. R., University of Pennsylvania, Philadelphia, Pa.
SCOTT, DR. ERNEST L., Columbia University, New York City, N. Y.
SCOTT, PROF. G. G., College of the City of New York, New York City, N. Y.
SCOTT, PROF. JOHN W., University of Wyoming, Laramie, Wyo.
SCOTT, PROF. WILLIAM B., 7 Cleveland Lane, Princeton, N. J.
SEVERINGHAUS, DR. AURA E., Department of Anatomy, College of Physicians and Surgeons, 630 W. 168th Street, New York City, N. Y.
SHULL, PROF. A. FRANKLIN, University of Michigan, Ann Arbor, Mich.
SHUMWAY, DR. WALDO, University of Illinois, Urbana, Ill.
SICHEL, DR. FERDINAND J. M., New York University, Washington Square College, New York City, N. Y.
SIVICKIS, DR. P. B., Pasto deze 130, Kaunas, Lithuania.
SNOW, DR. LAETITIA M., Wellesley College, Wellesley, Mass.
SOLLMAN, DR. TORALD, Western Reserve University, Cleveland, O.
SONNEBORN, DR. T. M., Johns Hopkins University, Baltimore, Md.
SPEIDEL, DR. CARL C., University of Virginia, University, Va.
STABLER, DR. ROBERT M., Department of Zoölogy, University of Pennsylvania, Philadelphia, Pa.
STARK, DR. MARY B., New York Homeopathic Medical College and Flower Hospital, New York City, N. Y.
STEWART, DR. DOROTHY R., Skidmore College, Saratoga Springs, N. Y.
STOCKARD, PROF. C. R., Cornell University Medical College, 1300 York Avenue, New York City, N. Y.
STOKEY, DR. ALMA G., Mount Holyoke College, South Hadley, Mass.
STRONG, PROF. O. S., College of Physicians and Surgeons, 630 West 168th Street, New York City, N. Y.
STUNKARD, DR. HORACE W., New York University, University Heights, N. Y.
STURTEVANT, DR. ALFRED H., California Institute of Technology, Pasadena, Calif.
SUMWALT, DR. MARGARET, University of Pennsylvania Medical School, Philadelphia, Pa.
SWETT, DR. FRANCIS H., Duke University Medical School, Durham, N. C.
TAFT, DR. CHARLES H., JR., University of Texas Medical School, Galveston, Tex.
TASHIRO, DR. SHIRO, Medical College, University of Cincinnati, Cincinnati, O.
TAYLOR, DR. WILLIAM R., University of Michigan, Ann Arbor, Mich.

TENNENT, PROF. D. H., Bryn Mawr College, Bryn Mawr, Pa.
THATCHER, MR. LLOYD E., Spring Hill, Tenn.
TRACY, PROF. HENRY C., University of Kansas, Lawrence, Kans.
TREADWELL, PROF. A. L., Vassar College, Poughkeepsie, N. Y.
TURNER, PROF. C. L., Northwestern University, Evanston, Ill.
TYLER, DR. ALBERT, California Institute of Technology, Pasadena, Calif.
UHLENHUTH, DR. EDUARD, University of Maryland, School of Medicine, Baltimore, Md.
UNGER, DR. W. BYERS, Dartmouth College, Hanover, N. H.
VAN DER HEYDE, DR. H. C., Galeria, Corse, France.
VISSCHER, DR. J. PAUL, Western Reserve University, Cleveland, O.
WAITE, PROF. F. C., Western Reserve University Medical School, Cleveland, O.
WALLACE, DR. LOUISE B., 523 Cowper Street, Palo Alto, Calif.
WARD, PROF. HENRY B., University of Illinois, Urbana, Ill.
WARREN, DR. HERBERT S., Department of Biology, Temple University, Philadelphia, Pa.
WARREN, PROF. HOWARD C., Princeton University, Princeton, N. J.
WENRICH, DR. D. H., University of Pennsylvania, Philadelphia, Pa.
WHEDON, DR. A. D., North Dakota Agricultural College, Fargo, N. D.
WHEELER, PROF. W. M., Museum of Comparative Zoölogy, Cambridge, Mass.
WHERRY, DR. W. B., Cincinnati Hospital, Cincinnati, O.
WHITAKER, DR. DOUGLAS M., Hopkins Marine Station, Pacific Grove, Calif.
WHITE, DR. E. GRACE, Wilson College, Chambersburg, Pa.
WHITING, DR. PHINEAS W., University of Pittsburgh, Pittsburgh, Pa.
WHITNEY, DR. DAVID D., University of Nebraska, Lincoln, Nebr.
WIEMAN, PROF. H. L., University of Cincinnati, Cincinnati, O.
WILLIER, DR. B. H., University of Rochester, Rochester, N. Y.
WILSON, PROF. H. V., University of North Carolina, Chapel Hill, N. C.
WILSON, DR. J. W., Brown University, Providence, R. I.
WITSCHI, PROF. EMIL, University of Iowa, Iowa City, Ia.
WOGLOM, PROF. WILLIAM H., Columbia University, New York City, N. Y.
WOODRUFF, PROF. L. L., Yale University, New Haven, Conn.
WOODWARD, DR. ALVALYN E., Zoölogy Department, University of Michigan, Ann Arbor, Mich.
YOUNG, DR. B. P., Cornell University, Ithaca, N. Y.
YOUNG, DR. D. B., University of Maine, Orono, Me.
ZELENY, DR. CHARLES, University of Illinois, Urbana, Ill.

OBSERVATIONS ON THE ERYTHROPHORES OF SCORPÆNA USTULATA

DIETRICH C. SMITH AND MARGARET T. SMITH

(From the Naples Zoölogical Station and the Department of Physiology, University of Tennessee, Memphis)

Among those fishes most commonly used in studies concerned with color changes the dominant pigment-bearing cell has been the melanophore, and as a consequence the most obvious chromatic responses in these animals have been conditioned largely by alterations in pigment distribution within these melanophores. Inevitably this has attracted more attention to this type of cell than to the xanthophores and erythrophores even though these yellow and red-bearing pigment cells might also play a part in the color changes of such fishes. There is, however, a fair amount of literature dealing with the reactions of erythrophores and xanthophores, enough to raise some interesting questions as to their control and behavior. A goodly part of this work has been devoted to a determination of the governing factors, particularly humoral, responsible for the contribution of the erythrophores to the nuptial coloration displayed by certain forms during their spawning season.

In the scorpian fish, *Scorpæna ustulata*, the dominant pigment-bearing cell is the erythrophore. Since this form could be obtained in numbers suitable for our purpose, a particularly favorable opportunity was given us to study erythrophore activity while at the Naples Zoological Station, where this investigation was done in the autumn of 1931. We wish to take this opportunity to express our appreciation for the privilege of using the Columbia-Woods Hole table at this institution, and also to express our gratitude and thanks to Professor R. Dohrn, the Director of the Station, for the courtesies which he extended to us during our stay in Naples.

As usually found in and about the waters of the Bay of Naples, the animal has a deep red color over the entire dorsal and lateral surface of the body, due to the presence of large numbers of erythrophores in the scales and in the deeper layers of the skin. When a scale is removed from the fish and examined microscopically, the plentitude of erythrophores is apparent, melanophores and xanthophores being scarce in comparison. Realizing the importance of erythrophores in contributing to the color of many tropical and sub-tropical fishes where the dominating tone is frequently red, it was thought desirable to investigate the

behavior of these cells in *Scorpæna ustulata,* both in the intact animal and in isolated scale preparations. Whenever convenient, observations were also made on the xanthophores and melanophores.

Experimental

The animals used in these experiments were all adults 20 to 30 cm. in length. When kept upon a black background they have a brilliant red color, but when placed upon white, this color fades to a pale pink. This fading requires anywhere from several hours to over a day to come to completion, a relatively slow process when compared with the rapidity of color changes in some other fishes. Similarly, when returned to black, the resumption of the deep red takes an equally long time. This raises the question as to whether or not these color changes are humorally controlled in the sense described by Giersberg (1930) for *Phoxinus,* where there is no evidence of a nervous control over the erythrophores. In *Scorpæna,* however, such evidence is not lacking, for if the sympathetic cord be severed at a point midway on the trunk in an animal adapted to a dark background and the fish be placed upon a white background, the portion of the body posterior to the cut retains its characteristic deep red color even after the portion anterior to the cut has paled. Such an experiment is of the type originally done by Pouchet (1876) and v. Frisch (1911, 1912) in demonstrating nervous control of the chromatophores. Furthermore, if the anterior end of the *Scorpæna* medulla be electrically stimulated when the animal is deep red, the whole body immediately pales, but in an animal where the sympathetics in the posterior trunk have been sectioned, stimulation of the medulla produces paling only in the skin anterior to the cut. The paling in all cases occurs with great rapidity (10 to 15 seconds) in marked contrast to its slow development when the fish is placed on a white background. There is then ample reason to believe that in *Scorpæna* the erythrophores not only possess nervous connections with a pigmentary motor-center in the medulla, but they are also capable of receiving and responding to impulses sent out from this center. The slowness with which the response to background changes develops is suggestive of some sort of neuro-humoral control of the type described by Parker (1932).

The presence of functional nervous connections in the erythrophores of *Scorpæna* places them in a group with those of *Crenilabrus pavo* and *Trigla corax.* These two forms, according to v. Frisch (1912), possess erythrophores and xanthophores with an innervation similar to that of the melanophores, as judged by the effects of electrical stimulation of

the pigment-motor center in the medulla. In *Scorpæna, Crenilabrus,* and *Trigla* the impulses are distributed to the chromatophores by way of the sympathetic system. Lode (1890), however, early reported the failure of the erythrophores of the trout to contract when the spinal cord or medulla were stimulated electrically, although such stimulation produced melanophore contraction. It was only on prolonged local stimulation that he observed any contraction of the erythrophores. Furthermore, Giersberg (1930) could find no evidence for a nervous control of the erythrophores and xanthophores of *Phoxinus,* the usual tests for nervous control such as stimulating the pigment-motor center in the medulla or cutting the sympathetics failing to evoke any positive response. Later Giersberg (1932) demonstrated that extirpation of the hypophysis in *Phoxinus* prevents all responses on the part of the erythrophores and xanthophores, thus giving added weight to his conclusion that erythrophore and xanthophore activity in *Phoxinus* was controlled solely through the activity of this gland. Fries (1931), working on *Fundulus heteroclitus,* a form possessing no erythrophores, demonstrated some measure of nervous control of the xanthophores, but he also concluded, in view of the differences in response between the xanthophores and melanophores, that a humoral mechanism of some sort was involved, acting to enforce the nervous mechanism or even at times possibly superceding it. Matthews (1933), however, observed in *Fundulus* the usual responses of the xanthophores to white and yellow background after hypophysectomy. In his operated animals the xanthophores contracted over white and expanded over yellow, as did those of normal unoperated fishes. The possibility of two different controlling mechanisms being present in the fishes in respect to lipophore activity, as suggested by Giersberg (1932), one nervous and the other humoral, is certainly worthy of serious consideration. In all probability a third type must also be considered, one where both mechanisms are present, one or the other assuming greater or less prominence as the case may be.

An extremely interesting phenomenon which many pigment cells exhibit under certain circumstances is the ability to show rhythmical pulsations after the proper treatment. Such pulsations, consisting as they do of a regular migration of the pigment granules in and out of the processes, were first observed by Ballowitz (1913*b*) in the erythrophores of excised skin from the trunk of *Mullus* when immersed in 0.75 per cent NaCl. This phenomenon is undoubtedly of the same nature as one described later by Spaeth (1916) in *Fundulus* melanophores. In this case isolated scales were immersed in N/10 $BaCl_2$ for about five minutes and then transferred to N/10 NaCl, where after a wait of

fifteen minutes or so the scale melanophores showed pronounced rhythmical pulsations. Methods other than treatment with $BaCl_2$ have also been described as producing pulsations in *Fundulus* melanophores (Smith, 1930; Yamamato, 1933). Isolated scale erythrophores of *Scorpæna,* when placed in N/10 NaCl, expand and remain so for about two to three hours until they begin to contract, resembling in this respect the melanophores of *Fundulus.* Furthermore if, again like the melanophores of *Fundulus,* the scales are first immersed in $BaCl_2$ and left there until all of the erythrophores are either completely or almost completely contracted (which usually occurs in about five minutes) and then returned to N/10 NaCl, the erythrophores will shortly begin a series of regular pulsations lasting for two or three hours. These pulsations seem to be of the sort described by Spaeth in *Fundulus* melanophores and probably, as is also the case in *Fundulus* melanophores, differ only from those seen by Ballowitz in *Mullus* in that the movements do not start spontaneously in NaCl.

Effects of Drugs on Isolated Scale Erythrophores

Isolated scale material from *Scorpæna* was found extremely well adapted for the study of the effects of drugs upon erythrophores. The scales lend themselves particularly well to such a study as they are easily removed from the body, are sufficiently transparent for microscopic work, and are of a favorable size, ranging from 2 to 4 mm. in width. Spaeth (1913) was the first to recognize the usefulness of such preparations in making direct microscopic observations upon the response of the chromatophores to various stimuli.

In testing the effects of drugs upon the isolated scale erythrophores of *Scorpæna,* the following procedure was adopted. First the scales were immersed in a solution made up of six parts of N/10 NaCl and one part N/10 KCl. Such a solution might or might not produce and maintain a condition of slight erythrophore expansion. If not, more NaCl or KCl was added to the solution, NaCl if the erythrophores were contracted, KCl if they were expanded, until a mixture was obtained which did maintain the erythrophores in the stellate state within the time limits of the experiment. Having attained a suitable combination of NaCl and KCl, the drug to be tested was added to the mixture to make whatever concentration was desired, a certain portion of the original having been set aside previously as a control. From time to time the condition of the erythrophores in the experimental solution was compared with that of the cells in the control. Such a procedure, while not entirely satisfactory, was necessary as the combined effects

of NaCl and KCl were variable within certain prescribed limits. But inasmuch as the drugs to be tested had powerful effects in either one direction or the other, the method was found suitable in determining whether a drug acted either to expand or contract the chromatophore. In any discussion of the changes in pigment distribution within the chromatophore it must be borne in mind that the pigment granules migrate in and out of permanently formed cellular processes (Ballowitz, 1913*a*; Matthews, 1931).

Nicotine

Scales with stellate erythrophores immersed in nicotine (Merck) solutions ranging from 1/100,000 to 1/10,000 showed first an immediate and pronounced expansion of all erythrophores. Eventually this expansion was replaced by the contracted or stellate condition, usually in about twenty minutes. This contraction persisted for at least three hours, the longest period that any single case was kept under observation. Between concentrations of 1/10,000 to 1/2,000 there was first a slight expansion (in one case at 1/2,000 no expansion whatever) which was followed in about twenty minutes by complete and lasting contraction. With stronger solutions (1/1,000–1/100) the higher concentration produced an immediate expansion which persisted without change, the lower concentrations (1/1,000) also leading to the same result although not so rapidly.

The initial effect then, regardless of strength, was one of expansion, followed in the case of weaker solutions by a contraction. This order, peculiarly enough, is just the reverse of what is obtained when nicotine is injected into the fish, such injections giving first a contraction, due presumably to an initial stimulation of the sympathetic ganglia, followed by an expansion, the result of an eventual peripheral sympathetic paralysis. Why the order should be reversed on the immersion of isolated scale erythrophores in nicotine solutions and whether the reversal is of any significance is difficult to say.

Physostigmine

Physostigmine acts to produce an expansion of the erythrophore pigment, an expansion which persists for at least one and one-half to two hours, the longest time any one scale was kept under observation. This expansion was observed in 0.0065 per cent and 0.013 per cent solutions. No other concentrations were tried. The expansion was at its maximum usually within ten or fifteen minutes after immersion. In these so-called expanded cells the pigment distribution in most cases presented

certain peculiarities, the pigment as a rule being mostly concentrated at the ends of the processes and in the central body. Since those parts of the processes between the tip and central body were filled only with a few scattered granules, the cell had the appearance of a central pigment mass surrounded by an outer pigment ring with an apparent empty space between. Close inspection, however, revealed sufficient pigment within the processes to outline them. But the appearance of the cells differed markedly from that presented when they were immersed in NaCl. In such a salt the pigment is evenly distributed throughout the processes and central body. "Ringed" erythrophores, as seen in the physostigmine solutions, when placed in a mixed NaCl and KCl solution again became stellate and when put in KCl immediately contracted. Such contracted erythrophores when transferred to NaCl expanded in the usual manner, the previous exposure to physostigmine in no way preventing a subsequent even distribution of the pigment. Not all of the scales placed in physostigmine showed the "ringed" type of expansion just described. In some cases the expansion appeared to be of the usual type with the pigment evenly distributed in the processes. In others the amount of pigment in the processes was relatively scant and the process consequently had a thin frayed-out look quite different from the well-defined and well-filled-out appearance shown in NaCl. In their responses to physostigmine, the melanophores were about the same as the erythrophores, except that as a rule the melanophore expansion tended to be better defined, less thin and less frequently "ringed."

Pilocarpine

Pilocarpine in concentrations ranging from 0.016 per cent to 0.04 per cent had essentially the same effects as physostigmine, producing in the majority of cells a thin or "ringed" expansion of both the erythrophores and the melanophores.

Atropine

Atropine in concentrations ranging from 0.025 per cent to 0.0065 per cent produced an immediate expansion of the erythrophores and melanophores which was rapidly converted into the "ringed" expansion of the type already described under physostigmine. The second effect appeared within two to ten minutes after the exposure to the drug and persisted for as long as the scales were left in the solution. When such atropinized scales were transferred to N/10 KCl within thirty to forty minutes after their initial immersion in atropine, an interesting reversal

of the usual response on the part of the chromatophores to KCl was seen. Ordinarily N/10 KCl immediately contracts and maintains contracted all of the chromatophores of isolated *Scorpæna* scales for at least two hours. Atropinized scales immersed in N/10 KCl showed the usual immediate contraction of their erythrophores and melanophores, but instead of staying contracted for an extended period as would be normally expected in such cases, these chromatophores quickly reversed their condition and rapidly assumed the state of maximal expansion, remaining so for at least two hours. This expansion usually occurred within two minutes after the transfer from atropine, although treatment with the weakest effective solutions of this drug required longer times—five minutes or so—before the re-expansion was established. In all scales the re-expanded erythrophores showed processes in which the pigment was evenly distributed. Control scales treated in precisely the same manner as the atropinized ones—except for the treatment with atropine—contracted in the usual manner in N/10 KCl, and stayed contracted for the usual two hours or so. We have then a clear and well-established case of the reversal of the effect of KCl brought on by previous treatment with atropine. To the best of our knowledge such a reversal has never been reported before. As mentioned previously, the reactions of erythrophores and melanophores were alike. The specificity of the effect as far as KCl is concerned is shown by the fact that when the scales are immersed in 1/100,000 adrenaline after atropine, the resulting contraction of the chromatophores is permanent. In other words, atropine does not reverse the effect of adrenaline, although it does reverse the effect of KCl. Atropinized chromatophores, expanded in KCl, when placed in 1/100,000 adrenaline immediately contracted and stayed contracted.

The atropine reversal of the effect of KCl is particularly interesting when considered in conjunction with the results of Spaeth and Barbour (1917), who found that adrenaline after ergotoxin produced an expansion instead of a contraction of the melanophores, a contraction which might or might not be lasting. The contracting effect of KCl, however, was unaffected, although its development was usually less rapid. In *Scorpæna*, however, the KCl effect upon the erythrophores and also upon the melanophores is reversed by atropine while the adrenaline effect is not changed. Spaeth and Barbour believed adrenaline to act upon the sympathetic endings in the chromatophore, while KCl acted upon the cell directly. Adrenaline apparently does act as they thought, but whether KCl is directly protoplasmic in its effect is another matter. In any event the results on *Scorpæna* erythrophores and on *Fundulus* melanophores show clearly that the contracting action of adrenaline and

KCl are not due to their action upon the same receptive mechanism within the cell.

The typical "ringed" expansion produced by atropine is also produced by pilocarpine and physostigmine and would indicate a direct protoplasmic effect upon the erythrophore rather than any action upon nerve endings present in the isolated scale preparations. In spite of this similarity in action, atropine is nevertheless separable from the other two drugs by virtue of its reversal of the effect of KCl, something which pilocarpine and physostigmine do not produce. Obviously atropine has some subtle action on the erythrophores different in nature from that of pilocarpine and physostigmine which is not disclosed by the character of the pigment distribution produced by these three drugs.

Acetyl-Choline

Solutions of acetyl-choline (0.1 per cent to 0.01 per cent) had no observable effect upon either the erythrophores or the melanophores of *Scorpæna ustulata.*

Cocaine

Cocaine in solutions of 0.1 per cent to 0.001 per cent produced contraction of the erythrophores and the melanophores in *Scorpæna ustulata.* There was, however, a pronounced tendency on the part of the melanophores to contract much more slowly than the erythrophores, the former contracting in about five minutes and the latter in about ten to twenty minutes, depending on the strength of the solution. These contractions lasted at least three hours. Scales with contracted chromatophores when returned to the N/10 NaCl–KCl mixture showed a re-expansion of their pigment cells to the stellate within twenty to thirty minutes. It is possible though not proven, that cocaine contracts the isolated scale erythrophores by virtue of its excitatory effect upon the peripheral sympathetic fibers.

Ethyl Alcohol

Scales with chromatophores in the stellate condition when placed in 10 per cent alcohol, usually show a pronounced expansion of all the pigment cells, the expansion occurring within the first few minutes after immersion. This in turn is replaced within the next five minutes by a concentration of the pigment within the central body. Within fifteen to thirty minutes, however, the pigment cells once more expand, this time to the "ringed" condition, in which they remain for at least three hours. In 5 per cent alcohol the same sequence of reactions is observed

except perhaps that the final expansion is longer delayed, often not occurring until almost one hour after the beginning of the experiment. In 1 per cent alcohol, however, there is in the majority of the cases no sign of any initial expansion; instead the primary response of the chromatophores is a contraction, oftentimes immediate or on occasion delayed for fifteen or twenty minutes. Having once contracted in 1 per cent alcohol, the pigment cells remain in that condition for three hours at least, the longest time any set of experimental scales was kept under observation. In no case was any sign of expansion evident within this period. One scale with stellate erythrophores did show a slight expansion when the scale was first placed in 1 per cent alcohol, but this expansion in no way approached the maximum and was replaced within fifteen minutes by a complete contraction. In most cases in 1 per cent alcohol the erythrophores contracted before the melanophores, and when the time for re-expansion had come the melanophores usually expanded before the erythrophores.

The "ringed" expansion in the stronger alcoholic solutions is suggestive of protoplasmic injury as it is with pilocarpine, physostigmine, and atropine; but the primary expansion and subsequent contraction are probably not direct effects. The possibility of explaining these responses, and also the responses to nicotine, by assuming an effect on the endings of two different types of nerve fibers within the erythrophores with varying thresholds of stimulation at once suggests itself. But it would be premature to do more than call attention to this possibility, even though there is evidence that the chromatophores of other forms, particularly the melanophores, possess a double innervation.

Effects on Pigmentary Responses of Injecting Drugs into the Abdominal Cavity

To check the action of drugs upon the chromatophores in isolated scale preparations, observations were also made upon the results of injecting these same drugs into the abdominal cavity of the fish. On the whole the results were unsatisfactory and inconclusive, as the drugs used were only slightly effective if at all, except in lethal doses.

Nicotine

A strong solution of nicotine (1 drop in 1 cc.) produced violent convulsions immediately followed by a pronounced paling. This, however, lasted for only a few minutes, the animal reddening again with the

approach of death, and by the time it was dead it was completely red. Hewer (1927) reports nicotine as first producing paling and then darkening when injected into the dab; all the chromatophores, lipophores as well as melanophores, reacting the same.

Physostigmine

One cc. of a 0.06 per cent to 0.013 per cent solution had no effect. With 2 cc. of 0.05 per cent to 0.065 per cent a reddening was observed. In such cases the animal usually died, always in a very red condition. Abolin (1926) found physostigmine to have no effect on the erythrophores of *Phoxinus*.

Pilocarpine

A 0.065 per cent to 0.15 per cent solution in 1 cc. doses produced some reddening, but not until one to two hours after the injection. Abolin (1926) observed no change in erythrophores of *Phoxinus* after pilocarpine injection.

Atropine

One cc. to 0.3 cc. of a 0.5 per cent to 0.05 per cent solution had no observable effect upon the color of the animal either on white or black backgrounds. However, with doses of 1 cc. of 1 per cent solutions there was a pronounced reddening, which in one case persisted for at least two hours. Osterhage (1932) observed in *Gasterosteus aculeatus* and *Rhodeus amarus* that the injection of atropine produced a pronounced reddening of the fins of the fish during the spawning season (May and June), but at other times of the year it only served to darken the animal. This was due presumably to the scarcity of erythrophores in the skin at times other than during the spawning season. Abolin (1926) reported atropine as being without effect on *Phoxinus* erythrophores.

Acetyl-choline

One cc. of 0.15 per cent solutions had no effect. Wunder (1931) observed in *Gasterosteus aculeatus* and *Rhodeus amarus* a slight reddening of the fins and belly following injection of acetyl-choline during the spawning season. Parker (1931) reports acetyl-choline as having a slight expanding effect on the chromatophores of *Fundulus*.

Cocaine

One cc. to 0.5 cc. of a 0.1 per cent to 0.25 per cent solution had no pronounced effects. In one case there was a slight paling, the meaning

of which was doubtful. In another instance with 1 cc. of 0.25 per cent the animal died, becoming slightly redder just before death.

Adrenaline

A solution of 1/2,000 produced (0.6 cc.) pronounced paling in about twenty minutes. Abolin (1925) reports adrenaline as having no effect on *Phoxinus* xanthophores and Giersberg (1930) also failed to observe any effect of adrenaline upon the erythrophores of this form, although he did see a contraction of the erythrophores of *Macropodus,* an Asiatic fresh-water form.

Hypophysis Extracts

Extracts of the posterior pituitary of fishes were made by removing the gland and macerating it in distilled water. This extract was filtered and the filtrate injected into *Scorpæna.* Extracts made from the hypophyses of the tuna fish, *Thynnus thynnus,* and from *Trigla* sp., on injection into *Scorpæna ustulata* produced a paling which lasted for several hours. The tuna extract was made by macerating one gland in 5 cc. of water and the *Trigla* extract consisted of one gland in 1 to 2 cc. of water. Attempts were made to prepare an extract from the hypophyses of *Scorpæna ustulata* which would produce a contraction of the erythrophores in the same species, but none were successful. The failure to get a potent extract probably followed a failure to use a sufficient number of glands in preparing the solution. But lack of material prevented the preparation of extracts stronger than the ones used (four hypophyses in 2 cc. of water). This makes it doubtful whether any hormone secreted by the *Scorpæna* hypophysis is responsible for any of the observed pigmentary changes.

In *Phoxinus,* Abolin (1925) and Giersberg (1930) report infundin as producing melanophore and lipophore expansion. One of us (D. C. S.) had occasion some time ago to try the effect of an extract of trout pituitary (posterior lobe) on *Phoxinus,* and found it to produce a pronounced contraction of the melanophores and an equally pronounced expansion of the erythrophores. Hewer (1925) had previously observed extracts of cod pituitary to contract the melanophores of *Phoxinus* and to expand the xanthophores and erythrophores, and Matthews (1933) found extracts of *Fundulus* pituitary to contract the melanophores and expand the xanthophores of isolated *Fundulus* scales. However, both Hewer and Matthews doubt whether any hypophysial hormone is responsible for the color changes observed in the forms on which they worked, and we have seen that this is also probably true in

the case of *Scorpæna*. Giersberg (1932), however, observed extirpation of the hypophysis in *Phoxinus* to abolish all color changes produced by the lipophores. Wunder (1931) and Osterhage (1932) saw an expansion of the erythrophores in the stickleback and bitterling following injection of commercial hypophysis extracts. Meyer (1931) observed contraction of the melanophores and expansion of the erythrophore in *Gobius* and *Pleuronectes* after hypophysis injection. In *Phoxinus, Fundulus, Gasterosteus, Rhodeus, Gobius,* and *Pleuronectes,* hypophysis extracts produce an expansion of the erythrophores or xanthophores, but in *Scorpæna,* fresh fish hypophyses extracts produce a contraction of the erythrophores. In those forms where expansion is the rule the erythrophores or xanthophores are relatively less active in comparison to the melanophores, and in the first four mentioned they are primarily associated with the production of nuptial coloration, except perhaps in *Fundulus,* and even in this form the xanthophores are probably most active during the spawning season. In *Scorpæna,* however, the erythrophores are the dominating pigment cells, the melanophores being subordinate in number and effect, and it is primarily the erythrophores which are responsible for the color changes the animal displays. It would seem as if in *Scorpæna* the erythrophores have assumed a different type of pigmentary activity than in the other forms mentioned.

It is perhaps well to remark here that in *Scorpæna* no qualitative difference between the responses of the erythrophores and the melanophores was ever seen. Within the same scale the erythrophores often reacted more rapidly than the melanophores, but the direction of the response was always the same. This again is in marked contrast to the condition found in some other forms where the melanophores and the erythrophores differ widely in the nature of their reactions. The xanthophores, however, are sharply differentiated from the erythrophores, particularly in respect to a greater sluggishness of behavior and a failure to pulsate in NaCl after $BaCl_2$.

Summary

1. Evidence is presented to show that the erythrophores of *Scorpæna* will respond to nervous stimulation.

2. Isolated scale erythrophores of *Scorpæna* will show rhythmical pulsations in N/10 NaCl after treatment with N/10 $BaCl_2$.

3. Isolated scale erythrophores of *Scorpæna* will show the following responses when immersed in the following drugs:

a. Nicotine in weak solutions (1/100,000–1/10,000) produces a primary expansion followed by contraction. Moderately strong solu-

tions (1/10,000–1/2,000) produce a slight expansion followed by contraction. Strong solutions (1/2,000–1/100) produce a permanent expansion.

b. Physostigmine (0.0065–0.013 per cent) produces a "ringed" expansion.

c. Pilocarpine (0.016–0.04 per cent) produces a "ringed" expansion.

d. Atropine (0.025–0.0065 per cent) produces a "ringed" expansion. After atropine, however, the usual lasting contraction produced by N/10 KCl persists only a minute or so and is replaced by a permanent expansion. There is no change in the contracting effect of adrenaline after atropine.

e. Cocaine (0.1–0.01 per cent) produces a contraction.

f. Alcohol in strong solutions (10–5 per cent) produces first an expansion then a contraction and finally a "ringed" expansion. Weak solutions (1 per cent) produce a persistent contraction.

g. Acetyl-choline (0.1–0.01 per cent) had no effect.

4. No relation was found between the effect of drugs upon isolated scale erythrophores and their effects upon the color of the fish after injection into the abdominal cavity.

5. Hypophysis extracts of the tuna and *Trigla* produce a pronounced and lasting paling (erythrophore contraction) when injected into the abdominal cavity of *Scorpæna ustulata*.

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THE PENETRATION OF THE BLUE AND RED COMPONENTS OF DAYLIGHT INTO ATLANTIC COASTAL WATERS AND ITS RELATION TO PHYTOPLANKTON METABOLISM

G. L. CLARKE AND R. H. OSTER

(From the Woods Hole Oceanographic Institution,[1] and the Laboratory of General Physiology, Harvard University)

The following observations were undertaken as a continuation of the investigation of the conditions of submarine irradiation in relation to the biology of marine organisms. The measurements reported here and previously (Clarke, 1933) on the penetration of daylight into Atlantic waters serve as a basis for a comparison of this region with others which are being investigated intensively (Atkins and Poole, 1933; Utterback, 1933; Utterback and Boyle, 1933; Richardson, 1933; Erikson, 1933; Pettersson, 1934; and Pettersson and Landberg, 1934). The present measurements were made at two stations in the Gulf of Maine, one in the deep central part and one on Georges Bank, and at four localities in the region of Woods Hole. At the stations in the Gulf of Maine the vertical distribution of the phytoplankton was investigated (Braarud, 1934), complete hydrographic observations were made, and the diurnal migrations of copepods during the entire periods were studied (Clarke, 1934). Detailed information on the irradiation present at various depths is valuable not only in its immediate application to these studies of the plankton but also as an essential part of an intensive study of the oceanography of the Gulf of Maine (*cf.* Rakestraw, Gran, and Waksman, 1933). The observations in the neighborhood of Woods Hole were made in Woods Hole Harbor, Buzzards Bay, Vineyard Sound, and off Gay Head—localities chosen as representing four typical environments in this region. It is hoped that these measurements of light penetration will be useful in relation to certain of the biological problems being studied at Woods Hole, particularly those dealing with ecological relationships (*cf.* Allee, 1934). Some measurements of the photosynthesis of diatoms in this region are reported at the end of this paper.

[1] Contribution No. 45.

Apparatus and Methods

The photoelectric method previously described (Gall and Atkins, 1931; Clarke, 1933) was used. For the measurement of the blue component of daylight (3460Å–5260Å) the same deck photometer was employed. For submarine measurements the gas-filled potassium (sensitized) photoelectric cell was used in the water-tight case equipped with the four-conductor cable and the internal shutter. This combination is designated in the tables as Photometer 1*a*. The high sensitivity of the gas-filled cell made it useful for measurements at the deeper levels, but more trouble with changes of emission—especially near the surface—was experienced than with the vacuum cell. The cell was always glowed before each reading and all the other precautions previously described were taken in these observations.

For measurements in the red region of the spectrum two Type PJ–14 vacuum cæsium on cæsium oxide cathode photocells were used (kindly furnished by the General Electric Co.) which have peaks of maximum sensitivity in the near ultra-violet and red regions of the spectrum (Fig. 1). One of these cells was mounted in a newly constructed deck photometer and the other in sea photometer case No. 2—this latter combination being designated in the tables as Photometer No. 2*b*. To limit the radiation reaching the cells, each was mounted under a Schott-Jena Type RG–1 glass filter, 5 cm. square and 2 mm. thick, which cut out all radiations shorter than 6000Å, and a water-cell 2 cm. in depth and containing a 1.5 per cent solution of $CuCl_2$ which absorbed radiations of wavelength longer than 7000Å (*cf.* Coblentz, 1911). The filters were placed against the inside surface of the photometer window, the spaces between the filters and between the upper filter and the window being filled with Canada balsam to minimize reflection loss at the glass faces. When examined spectroscopically the duplex filters were found to have a sharp cut-off at 6000Å and a graded cut-off at 7000 to 7100Å with approximately 3 per cent transmission at 7000Å.

The spectral sensitivities of the cæsium photocells without the diffusing disc and filters were determined by the General Electric Research Laboratory under the direction of Dr. L. R. Koller (Fig. 1). The photocells mounted in the photometer cases under the filters were standardized for the spectral range 6000Å–7000Å by the same method as that employed for the blue-sensitive photometers (Clarke, 1933).

No changes in emission due to "photoelectric fatigue" were observed in moderate steady light in using the cæsium cells, but after continued exposure to bright sunlight on the deck of the ship decreases in the sensitivity of these cells were found amounting to 14 per cent. The

presence of " dark currents " in the deck photometer required frequent readings of the response with the photometer shutter closed during a series of observations. All measurements below the surface have been corrected for " external reflection " and " internal reflection " by multiplying by the factor 1.09 as before.

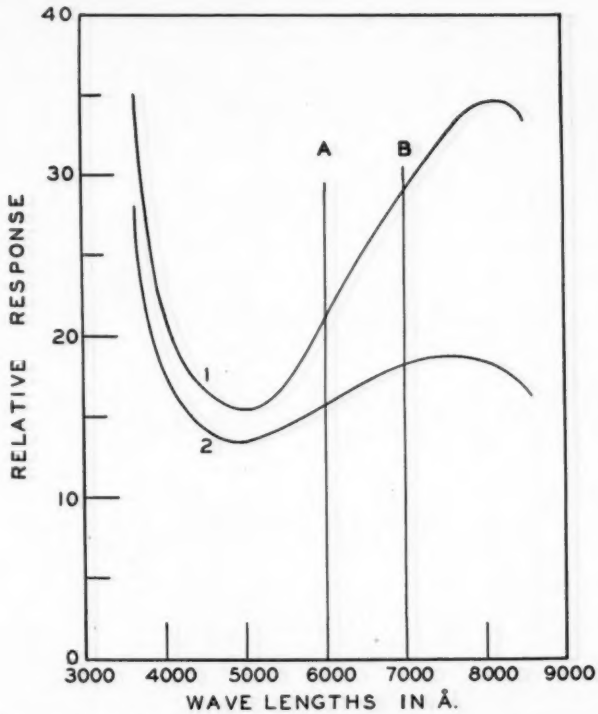

FIG. 1. Comparison of spectral sensitivities of red-sensitive photocells. (General Electric Research Laboratory.) Curve 1. No. 31,613. Cæsium on cæsium oxide—sea cell. Curve 2. No. 31,797. Cæsium on cæsium oxide—deck cell.

Each curve represents the relative response to an equal energy spectrum of the photocell alone.

In practice the cells are considered to be sensitive to the range between the vertical lines *A* and *B* as limited by the red RG–1 filter and the $CuCl_2$ filter cell.

OBSERVATIONS

The observations made on the penetration of blue and red light are summarized in Tables I and II. Curves showing the percentage of the

TABLE I

Summary of Observations in the Gulf of Maine

	Blue (3460Å–5260Å)			Red (6000Å–7000Å)			
Series No.	201	202	203	204	205	206	207
Station No.	1722	1722	1723	1722	1722	1723	1723
Lat. N.	42°50′	42°50′	41°19′	42°50′	42°50′	41°19′	41°24′30″
Long. W.	67°05′	67°05′	66°40′	67°05′	67°05′	66°40′	66°42′30″
Date, 1933	7/15	7/16	7/17	7/15	7/16	7/17	7/18
Local sun time (24-hr. scale)	1425–1515	1230–1440	1200–1410	1230–1340	1030–1200	1430–1535	1210–1325
Av. altitude of sun	48°	59°	63°	65°	64°	47°	56°
Photometer No	1a	1a	1a	2b	2b	2b	2b
Av. transmissive exponent, *k*, over depth studied	0.11	0.11	0.14	0.31	0.32	0.35	0.34
Irrad. at depth of 1 meter (% surface light)	77	89	64	43	53	52	46
Depth at which irrad. is reduced to 1%, *meters*	40	34	26	12	12	11	12
Range of irrad. on deck during series *microwatts/cm.*^{2}	4,500–8,200	5,600–8,600	11,900–13,400	13,900–12,200	10,700–8,700	11,000–5,600	10,000–11,400
Sea (Douglas sea scale)	Slight	Smooth	Smooth heavy swell	Slight	Smooth	Smooth heavy swell	Slight heavy swell
Sky	Many clouds	Over-cast, fog	Clear, fog	Few clouds	Over-cast, fog	Clear, fog	Clear, fog

TABLE II

Summary of observations in the Woods Hole region. Woods Hole harbor, Series 122 and 211: bottom at 23 meters, tide flooding, *i.e.* water flowing in from Buzzards Bay. Off Gay Head, Series 208 and 212: bottom at 30 meters, current running west until 1537. Buzzards Bay, Series 209 and 213: bottom at 15 meters, high tide at New Bedford at 0920. Vineyard Sound (halfway between Nobska Point and West Chop), Series 210 and 214: bottom at 22 meters, current running east until 1500.

	Blue (3460Å–5260Å)				(Red (6000Å–7000Å)			
Series No.	122	208	209	210	211	212	213	214
Location	W. H. Harbor	Off Gay Head	Buzzards Bay	Vineyard Sound	W. H. Harbor	Off Gay Head	Buzzards Bay	Vineyard Sound
Lat. N.	41°31′	41°20′	41°33′	41°30′	41°31′	41°20′	41°33′	41°30′
Long. W.	70°40′	70°57′	70°42′25″	70°36′	70°40′	70°57′	70°42′25″	70°36′
Date	Aug. 22 1932	Sept. 2 1933	Sept. 6 1933	Sept. 8 1933	Aug. 30 1933	Sept. 2 1933	Sept. 6 1933	Sept. 8 1933
Local sun time (24-hr. scale)	1339–1429	1320–1445	1011–1210	1040–1145	1410–1540	1150–1305	1215–1350	1310–1450
Av. altitude of sun	50°	47°	54°	54°	40°	56°	53°	46°
Photometer No.	1	1a	1a	1a	2b	2b	2b	2b
Av. transmissive exponent, *k*, over depth studied	0.49	0.27	0.51	0.36	0.62	0.37	0.61	0.59
Irrad. at depth of 1 meter (% surface light)	48	79	50	47	29	39	43	42
Depth at which irrad. is reduced to 1%, *meters*	8	16	10	12	6	10	7	8
Range of irrad. on deck during series, *microwatts/cm.*^{2}	11,800–12,400	14,000–11,000	12,000–9,000	14,000–13,000	7,950–5,800	14,250–13,400	11,050–9,400	11,500–10,200
Sea (Douglas sea scale)	Calm	Smooth to slight	Smooth to slight	Smooth	Calm	Smooth to slight	Smooth	Slight
Sky	Clear	Clear	Clear	Clear	Clear	Clear	Clear	Clear

surface light reaching the various depths for all the series appear in Figs. 2 to 5. Considering first the penetration of blue light, we find that the average transparency at Station 1722 in the Gulf of Maine is slightly greater than that at Station 1053 (Clarke, 1933[2]). This is to be expected since Station 1722 is nearer the open ocean. The average transparency on Georges Bank (Station 1723), where the shallower water may be stirred down to the bottom, is about the same as at Stations 1285,

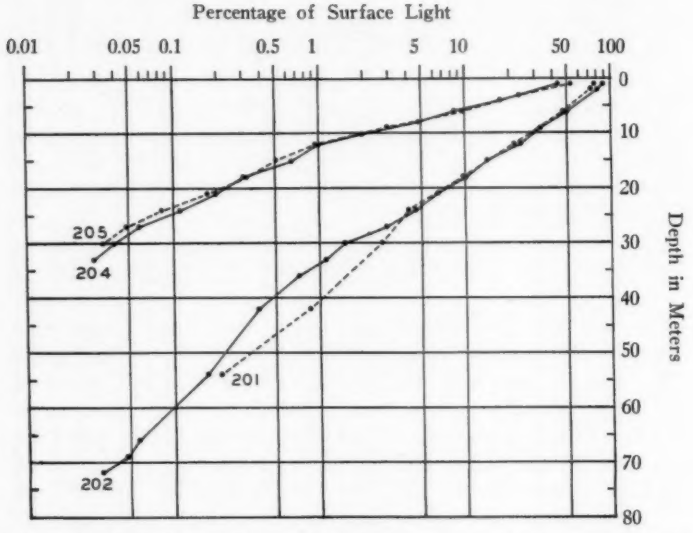

FIG. 2. Relation between depth and irradiation expressed as a percentage of the light just over the surface (logarithmic scale). Series number indicated at the end of each curve.

Station 1722: Gulf of Maine, deep basin.
Series 201 and 202—blue light.
Series 204 and 205—red light.

1286 and 1287 (previously reported) in deeper water but much nearer shore. The transparency of the water for blue light is from 2 to 4½

[2] The following corrections should be made in "Average altitude of sun" in Tables II and III, Clarke, 1933 Biol. Bull., vol. 65, p. 317.

TABLE II

Series No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Av. altitude of sun	58°	54°	58°	61°	62°	64°	65°	65°	62°	61°	24°	67°	54°

TABLE III

Series No.	13	112	113	114	115	116	117	118	119	120	121	122
Av. altitude of sun	54°	62°	1°	28°	37°	41°	68°	6°	22°	58°	57°	50°

times lower in the Woods Hole region than in the Gulf of Maine. The water of Buzzards Bay (Series 209) was found to have a slightly greater average transmissive exponent than the water of Woods Hole Harbor (Series 122). However, it is the turbidity of the water layer just over the bottom in Buzzards Bay which reduces the average transparency for the whole series, the upper strata being somewhat more transparent. In Woods Hole Harbor this situation is just reversed, the surface water being slightly less transparent than the deeper strata. The result is

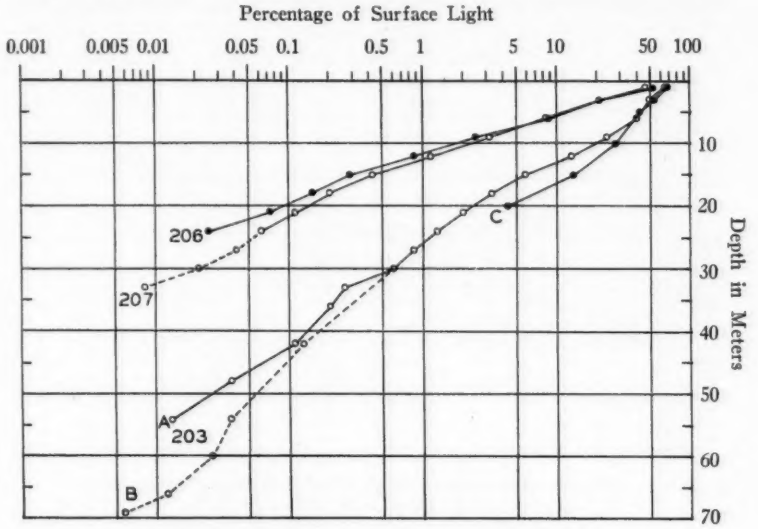

FIG. 3. Station 1723. Georges Bank.
Series 203—blue light.
a. Regular measurement.
b. Measurement of deeper strata with opal glass removed from photometer.
c. Measurement of upper strata repeated following day.
Series 206 and 207—red light. Broken portion of curve 207 represents measurement with opal glass removed.

that daylight penetrates to a depth of 10 meters in Buzzards Bay before it is reduced to 1 per cent of its surface value whereas a corresponding reduction has taken place in the Harbor at 8 meters. The water off Gay Head (Series 208) is about half as clear as that characterizing the Gulf of Maine, and the transparency in Vineyard Sound (Series 210) is intermediate between that found off Gay Head and in Buzzards Bay. The Gay Head and Vineyard Sound regions correspond rather closely to the San Juan Archipelago in transparency to blue light (Utterback and Boyle, 1933).

The average transparency for red light in the deeper part of the Gulf of Maine (Station 1722) is slightly higher than that found by Atkins and Poole (1933) off Rame Head, England, although there is a closer agreement when only the range from 1 to 10 meters is used for comparison. On Georges Bank (Station 1723) the transparency for red light falls between that found off Rame Head and in Whitsand Bay, for the range 1 to 5 meters, and is lower than at Station 1722. The penetration of red light into the water off Gay Head (Series 212) is only slightly lower than on Georges Bank and is higher than would be ex-

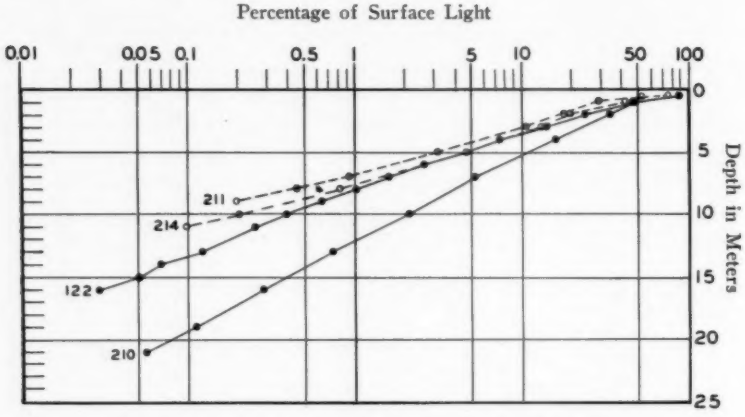

FIG. 4. Series 122. Blue light. Woods Hole Harbor.
Series 210. Blue light. Vineyard Sound.
Series 211. Red light. Woods Hole Harbor.
Series 214. Red light. Vineyard Sound.

pected for the locality. The waters of Vineyard Sound (Series 214), Buzzards Bay (Series 213), and Woods Hole Harbor (Series 211) are all very similar in transparency, but there is a slight increase in turbidity in the order mentioned. The transparency is approximately one-half that found at Station 1722.

In some of the series the transmissive exponent in the red decreases with depth. This is especially evident at Stations 1722 and 1723 and to a lesser extent in Series 212 where the depth of the water allows for some "layering." Atkins and Poole (1933) found similar results for certain observations in British waters, and Utterback and Boyle (1933) for measurements made in the waters of the San Juan Archipelago. Irradiation in the deeper layers is probably at the shorter wave-

lengths so that the energy reaching the photocell through the Schott RG–1 filter would be at or near to 6000Å, *i.e.* in the orange.

A comparison of the energy in the blue and red regions of the sun's spectrum is given in Fig. 6 as measured on the deck of the ship over the period of daylight on July 16, 1933 at Station 1722. For the spectral limits measured the agreement in the amount of energy is close. Since the sky during the period covered was overcast with some fog, the red radiation was probably transmitted more readily than the blue due to more scattering at the shorter wave-lengths. Scattering of blue light may also help to explain the close agreement at sunset when the red component would normally have the higher value.

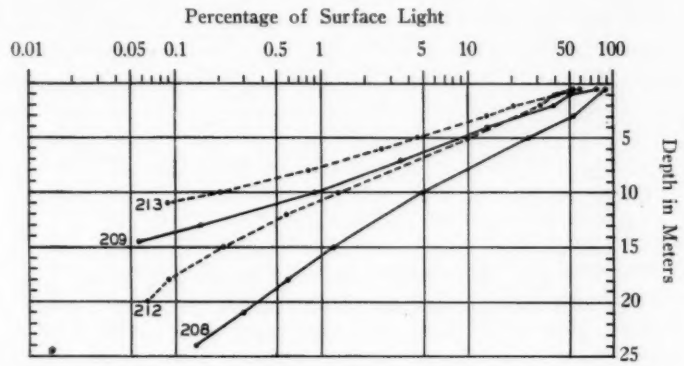

Fig. 5. Series 208. Blue light. Off Gay Head.
Series 209. Blue light. Buzzards Bay.
Series 212. Red light. Off Gay Head.
Series 213. Red light. Buzzards Bay.

DISCUSSION

The curves for the penetration of both blue and red light show marked differences in their degree of irregularity. Thus the curves for the Gulf of Maine are quite jagged in comparison with those for the Woods Hole region. This may be due in part to the much rougher condition of the sea experienced in the Gulf of Maine. It seems probable, however, that a more important factor is the thorough stirring caused by the very strong tidal currents of the Woods Hole region. Temperature measurements (such as those in Table III) show that the water is very nearly homogeneous from top to bottom. The irradiation measurements obtained in Vineyard Sound (*e.g.* Series 210), famous for its strong tide, fall on a remarkably straight line. Moreover,

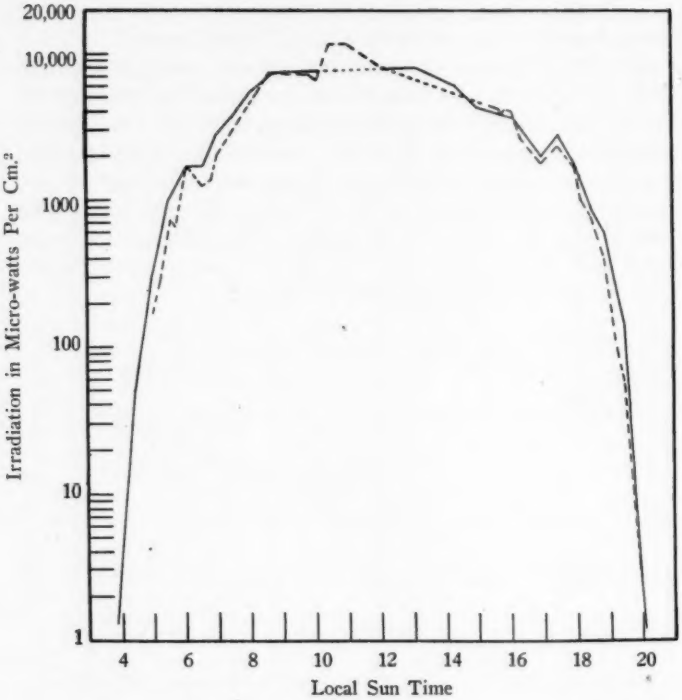

FIG. 6. Comparison of the blue and red components of daylight measured on deck during July 16, 1933 at Station 1722. Solid line indicates blue light, broken line indicates red light.

this regularity indicates that the method of measurement is highly reliable and that the irregularities in the values obtained at other localities are not to be assigned to fluctuation of the photometers.

If changes in the slopes of the curves really are caused by water layers of different transparency, it is difficult to understand why changes in the blue curve are not represented by corresponding changes in the red curve for the same locality, and vice versa, since the two sets of measurements were ordinarily taken within two hours of each other. Examples of such lack of conformity are seen, particularly, in the Gulf of Maine Series. The presence in different water layers of material having a highly selective effect on light would account for these irregularities, but the actual existence of such material has yet to be demonstrated. The differences between the three segments (*a*, *b*, and *c*)

of Series 203 certainly indicate a great lack of homogeneity in this case.

The relative clearness of the bodies of water considered is the same whether arranged in order of average transparency to blue light or to red light except for the Woods Hole Harbor and Buzzards Bay series. The transmissive exponent for blue light is slightly higher in Buzzards Bay than in Woods Hole Harbor, whereas the reverse is true for red light. However, the transparency of the harbor water is very close to that of the Bay water in both cases, and for blue light at least it is distinctly different from that of Vineyard Sound. This is in agreement with the fact that when the tide is flowing into the harbor, the water is derived chiefly from Buzzards Bay (*cf.* Fish, 1925).

Red light is absorbed faster than blue light in all of these bodies of water, but it is significant to note that the ratio of the transmissive exponents is not constant. The ratios of the exponents for red light to the exponents for blue light follow:

Station 1722	2.9
Station 1723	2.5
Off Gay Head	1.4
Vineyard Sound	1.6
Woods Hole Harbor	1.3
Buzzards Bay	1.2

It is seen that, in general, as the water becomes more turbid the difference between the penetration of blue and red light becomes less. This would seem explicable on the basis of the greater scattering effect of increasing amounts of suspended particles on radiation of short wave-length than on radiation of long wave-length. The ratio of the exponents for red and blue light in the Gulf of Maine is about the same as in Whitsand Bay (Atkins and Poole, 1933). The ratio in the Woods Hole region is similar to that in the San Juan Archipelago (Utterback and Boyle, 1933).

Note on the Metabolism of the Phytoplankton

Of previous investigations on the photosynthesis of aquatic plants in relation to the spectral distribution of the irradiation (*cf.* Klugh, 1930), few deal specifically with the marine phytoplankton. It is therefore difficult to correlate the present measurements of the penetration of light with the vertical distribution and metabolism of diatoms, etc. However, since photosynthesis is of primary significance in the economy of the sea, it seems important to report such observations as are available. Laboratory experiments indicate that intensity is more important than color for the photosynthesis of diatoms, but since chromatic adapta-

tion occurs, it is necessary to know the spectral composition as well as the total intensity of the irradiation present (Stanbury, 1931). Klugh (1930) concludes that certain pigments present in the cell may act as photo-sensitizers for the chlorophyll in plants growing at depths lower than the first few meters. Schreiber (1927) exposed various species of diatoms and dino-flagellates to general radiations and showed that the optimum radiant energy for growth differed widely in different forms.

On August 16 and August 22, 1932, Dr. H. H. Gran carried out measurements of the metabolism of diatoms in Woods Hole Harbor at the same time that the observations on the penetration of blue light were being made (Series 120, 121, and 122). Comparable values for red light may be obtained from Series 211. For the experiments sea water with the contained diatoms was taken from Vineyard Sound and placed in glass-stoppered bottles of 200 cc. capacity. The bottles were placed horizontally in shallow wire baskets and these were lashed at 3-meter intervals to a weighted rope hung from the south side of the anchored boat. Each basket contained two bottles fully exposed to light, and four of the baskets contained in addition one bottle which was completely covered with black cloth, thus providing an opportunity to measure respiration in the absence of photosynthesis. The oxygen present in two control bottles was measured, using the Winkler method at the beginning of the exposure period, and similar measurements were made on all the other bottles at the end. The bottles were exposed at their respective depths for about 4 hours during the middle of the day.

The experiments were not intended to yield more than approximations and the results are somewhat irregular. However, the location of the depth at which the oxygen consumed in respiration is just balanced by the oxygen evolved in photosynthesis (*i.e.* the "compensation point") seems quite definite. The results of the experiment on August 22 are presented in Table III, where it is seen that the bottles at the 3-meter and 6-meter levels showed an increase in oxygen whereas those at greater depths showed a decrease. Since on August 16 the shallowest bottle was placed at 8 meters and all the bottles showed a decrease of oxygen content at the end of the experiment, we may place the "compensation point" for Woods Hole Harbor at about 7 meters. At this depth the average irradiation present during the experiment was as follows:

Blue	192 microwatts/cm.^{2}
Red	108 microwatts/cm.^{2}

On June 1, 1934, Dr. A. C. Redfield carried out similar measurements, using the same methods, while the "Atlantis" was anchored at

Station 2212 in the Gulf of Maine 40 miles east of Cape Cod. A diatom haul made on Georges Bank was allowed to stand for 2 days and the suspended material decanted off and diluted. Bottles containing this diatom suspension were placed at 10-meter intervals down to 50 meters and left for 7 hours. When the tide was running strongly the bottles

TABLE III

Metabolism of diatoms in Woods Hole Harbor. Exposure period: 1002–1427, August 22, 1932. Range of irradiation on deck during exposure period: blue (3460Å–5260Å): 12,400–11,600 microwatts/cm.²; red (6000Å-7000Å): 12,000-11,200 microwatts/cm.²* Oxygen present before exposure: 6.74 cc./liter. Oxygen present in covered bottles at end of period: 6.64 cc./liter.

Depth	Temp.	Average irradiation blue	Average irradiation red	Oxygen after exposure	Change in oxygen
meters	° *C.*	*microwatts/cm.²*	*microwatts/cm.²*	*cc./l.*	*cc./l.*
3	21.40	1700	1240	7.06	+0.34
6	21.39	370	197	6.81	+0.07
9	21.33	98	23	6.72	−0.02
12	21.27	27	3	6.71	−0.03
15	21.26	7.2	—	6.73	−0.01
18	21.27	—	—	6.67	−0.07

*This value assumed from information obtained a year later on the ratio of the blue and red components of daylight.

were brought slightly nearer the surface, as is indicated in Table IV. The results are satisfactorily consistent and show that photosynthesis took place down to at least 40 meters. The progressive diminution of oxygen produced from the surface downwards is a result both of the decreased irradiation and of the lowered temperature. The amount of oxygen consumed in respiration likewise diminished with depth and is correlated with the temperature. The "compensation point" occurred at 30–24 meters.

Since no measurements of irradiation were made during these observations, it is impossible to make an exact comparison of the results with those obtained in Woods Hole Harbor. However, it is worth pointing out that in the Gulf of Maine the water is roughly three times as transparent as in Woods Hole Harbor and the "compensation point" lies about three times as deep. Marshall and Orr (1928) found the "compensation point" in Loch Striven to occur between 20 and 30 meters, but a satisfactory comparison with their observations is similarly difficult because of the different methods used. The calculation of light intensity for Loch Striven made by Atkins and Poole (1933) cannot readily be compared with ours since the values arrived at are for irradia-

tion over the whole spectrum throughout the day and ours are concerned with two limited regions of the spectrum for a few hours. It appears, however, that the water of Loch Striven, like the water in the Gulf of Maine, is somewhat more than three times as transparent as the water in Woods Hole Harbor and the "compensation point" for the whole day lies correspondingly deeper.

TABLE IV

Metabolism of diatoms in the Gulf of Maine. Exposure period: 0444–1354, June 1, 1934. Sky: cloudy, becoming clear and later overcast. Sea: smooth, moderate swell.
Oxygen present before exposure: 7.80
7.83
7.83

av. 7.82 cc. per liter.

Depth	Temp.	Condition of bottles	Oxygen after exposure	Change in oxygen	Oxygen produced
meters	° *C.*		*cc./l.*	*cc./l.*	*cc./l.*
0	10.75	covered	7.11	−0.71	
		exposed	9.42	+1.60	+2.31
		"	9.44	+1.62	+2.33
10–8	10.60	covered	7.32	−0.50	
		exposed	8.40	+0.58	+1.08
		"	8.44	+0.62	+1.12
20–16	10.0	covered	7.42	−0.40	
		exposed	8.07	+0.25	+0.65
		"	8.02	+0.20	+0.60
30–24	6–9	covered	7.61	−0.21	
		exposed	7.81	−0.01	+0.20
		"	7.72	−0.05	+0.16
40–32	5–6	covered	7.54	−0.28	
		exposed	7.66	−0.16	+0.12
		"	7.71	−0.11	+0.17
50–40	4–5	covered	7.61	−0.21	
		exposed	7.59	−0.22	+0.01
		"	7.64	−0.18	−0.03

From the samples of phytoplankton collected in July, 1933 at Stations 1722 and 1723 and subsequently subjected to a quantitative study by Mr. T. Braarud (1934) it was found that the dominant form as to numbers at both stations was the coccolithophoride *Pontosphæra Huxleyi*, which reached its maximum at approximately 18 meters at Station 1722

and at near 14 meters at Station 1723. Braarud (1934) states that the production of *Pontosphaera* seems to have been confined to the layer in which the temperature was higher than 10° C. At these depths where the maximum of *Pontosphæra* occurred the blue light was reduced to 9.9 per cent and 11.4 per cent respectively and the red light to 0.31 per cent and 0.50 per cent respectively. Taking 13,000 and 11,000 microwatts/cm.^{2} for approximate values of blue and red irradiation at the surface at noon on a clear day, we obtain light intensities of 1290 and 1480 μW/cm.^{2} for the blue and of 34 and 55 μW/cm.^{2} for the red for these depths. It will be noted that the blue irradiation is greater and the red irradiation less than that found at the "compensation point" in Woods Hole Harbor. However, no conclusions can be drawn from these facts as yet since the effect of the different parts of the spectrum on photosynthesis and the influence of other environmental factors are so poorly understood.

It is of interest to inquire whether the phytoplankton population has an appreciable effect on the reduction of light with depth (*cf.* Atkins and Poole, 1933). No sharp changes in the slopes of the curves occur at the levels of maximum abundance of *Pontosphæra.* The diatom, *Guinardia flaccida,* similarly has no noticeable influence upon the transparency. This species, being about fifty times larger than *Pontosphæra,* is the dominant form in respect to bulk at Station 1723 and occurs in maximum numbers at 30 meters.

It is evident that the whole question of the relationship between submarine irradiation and the metabolism of the phytoplankton must be much more thoroughly investigated both in the laboratory and at sea. In particular, for an understanding of the biology of Atlantic coastal waters we lack measurements of the penetration of the green and yellow parts of the spectrum. We now have a general idea of the vertical distribution of the plankton in these waters and of the conditions of submarine irradiation in respect to blue and red light.

Summary

1. Photoelectric measurements of blue and red submarine irradiation were made at two stations in the Gulf of Maine and at four localities in the Woods Hole region. The same apparatus was used as before (Clarke, 1933) with the addition of two cæsium on cæsium oxide photocells mounted under Schott-Jena RG–1 filters and $CuCl_2$ water-cells which limited the radiation received to 6000Å to 7000Å.

2. The bodies of water considered may be arranged in order of decreasing transparency to both blue and red light as follows: (*a*) the

deep basin of the Gulf of Maine, (*b*) Georges Bank, (*c*) Off Gay Head, (*d*) Vineyard Sound, (*e*) Buzzards Bay and Woods Hole Harbor. The ratios of the transmissive exponents for red light to those for blue light in each locality vary from 2.9 to 1.2, the higher values being characteristic of clearer water.

3. These measurements are discussed in relation to the local hydrographic conditions, and are compared with observations made in the Plymouth region and in the San Juan Archipelago.

4. Measurements made by Dr. H. H. Gran on the metabolism of diatoms in bottles suspended at different depths in Woods Hole Harbor indicated that the " compensation point " was located at a depth of about 7 meters. Similar measurements carried out by Dr. A. C. Redfield gave a depth of 24–30 meters for the " compensation point " in the Gulf of Maine.

5. Observations reported by Mr. T. Braarud (1934) on the vertical distribution of the phytoplankton at the same stations in the Gulf of Maine are discussed in relation to the penetration of light.

The authors desire to express their thanks to Mr. R. T. Montgomery for his kind assistance in the measurements made at sea.

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A NOTE ON THE PHYTOPLANKTON OF THE GULF OF MAINE IN THE SUMMER OF 1933

TRYGVE BRAARUD

(From the Woods Hole Oceanographic Institution,[1] Woods Hole, Mass.)

The rather scanty quantitative data on the phytoplankton distribution in the Gulf of Maine in summer are mostly from the western part, collected during 1931–32 for the International Passamaquoddy Fisheries Commission (Gran and Braarud, 1934). In addition, Gran (1933) studied the phytoplankton distribution in the summer of 1932, from material collected at several stations. The phytoplankton described in this paper was obtained from water bottle samples taken by Clarke at two stations in the Gulf of Maine during his work on the light conditions in the water and on the vertical migration of the zoöplankton. These stations represent much the same part of the Gulf as do two of the stations from which the 1932 material was collected. The 1932 observations were made on August 3–4, while those in 1933 occurred about two weeks earlier in the season, on July sixteenth and eighteenth. The present collections supplement our knowledge of the distribution of phytoplankton in this region and also provide data for the study of the relationships between the plant population and the penetration of daylight into the water. Since the latter aspect of the work is discussed by Clarke and Oster (1934) for these stations, I shall confine myself to a short account of the distribution of the phytoplankton. The question of the importance of diatoms in light absorption has been discussed by Poole and Atkins (1929) and by Marshall and Orr (1930).

The surface layers at Station 1722 (in the eastern part of the Gulf of Maine) consisted of presumably local water of low salinity (32.07 to 32.19‰), while at Station 1723 the uppermost layer was of Atlantic origin, having a salinity between 33.12 and 33.54‰. (See Table III.) In spite of this conspicuous difference in the characteristics of the water, the phytoplankton communities at the two stations had much the same character. The form numerically dominant at both localities was the coccolithophoride *Pontosphæra Huxleyi*, which was abundant at a depth of 1 to 25 or 30 meters. At both stations the maximum number was found at an intermediate depth (15, 18 meters), and the production of *Pontosphæra* seems to have been confined to the

[1] Contribution No. 46.

layer in which the temperature was more than 10° C. This is corroborated by the oxygen distribution, as the layer in which there was supersaturation of oxygen is the same as that in which the numbers of *Pontosphæra* were high. The only exception is Station 1723, where at 25 meters 30,000 cells of *Pontosphæra* per liter were found and yet there was no supersaturation in oxygen. Here the temperature was only 8.67° C. The general distribution of *Pontosphæra Huxleyi* seems clearly to indicate that it is a moderately thermophile form which reproduces very slowly at temperatures lower than 7° C. (Gran, 1930, p. 32). It seems probable, therefore, that this population at 25 meters was the result mainly of the sinking of the abundant population of *Pontosphæra* from the layers above (see Table III), and that the local production of *Pontosphæra* at this depth was small on account of the low temperature and the poor light conditions.

At Station 1723 (on Georges Bank) diatoms were fairly abundant, but only two species were recorded in any great numbers. In the upper warm layer *Detonula confervacea* occurred in small quantities in every sample, with a maximum number of 1,300 cells per liter at 15 meters—the same depth at which the maximum number of *Pontosphæra* occurred. *Guinardia flaccida* was distributed mainly below 20 meters, with a maximum of 5,300 cells per liter at 30 meters. This species has previously been recorded as a form which commonly, if not always, has occurred in the waters over the Bank (Bigelow, 1926; Gran, 1933). The *Ceratia* must have been very scarce at Station 1723, since in the centrifuged samples only a few specimens of *Ceratium longipes* and *C. tripos* were recorded. A colorless flagellate (*Bodo marina?*) was common at all depths.

At Station 1722 very few diatoms were observed, and no forms except *Pontosphæra Huxleyi* occurred in great numbers. The *Ceratia* were as scarce as at Station 1723. The *Guinardia* population was absent here, or at least present in numbers too small to be recorded in the volume of water which was centrifuged. Except for the *Guinardia*, the communities were very much the same in the two localities, but quantitatively poorer at Station 1722.

Although Gran's observations (1933) were not made in exactly the same localities as the present ones, an approximate comparison may be made between the summer vegetation at Georges Bank and in the eastern part of the Gulf of Maine in the years 1932 and 1933. The difference in time of season, viz., two weeks, is presumably of no great importance, since the conditions do not seem to change quickly at this time of the year. In the Gulf proper, a remarkable difference between the two years is found in the quality of the phytoplankton. In 1932 di-

TABLE I

Station 1722, July 16, 1933. Latitude, 42°50′ N, Longitude, 67°05′ W.

Depth in meters	1	6	12	18	30	42	54	66	78
Temperature, ° C.	13.17	13.04	12.52	11.39	10.46	8.50	7.01	6.59	5.77
Salinity, ‰	32.07	32.07	32.09	32.11	32.19	32.42	32.63	32.75	32.80
Oxygen, cc. per liter	6.48	6.41	6.65	6.87	6.85	6.60	6.48	6.42	6.20
Oxygen, % saturation	107	105	108	109	107	99	94	92	88
PO_4', mg. per cu.m	29	29	29	29	30	55	60	66	78
NO_3', mg. per cu.m	11	13	9	8	11	55	80	85	104
Number cc. examined	10	10	10	10(50)	10(50)	10	10	50	50
Phytoplankton, cells per liter:									
Chætoceros sp.	—	—	—	—	—	—	—	20	—
Corethron hystrix	—	—	—	—	—	—	—	20	—
Detonula confervacea	200	100	—	200	100	—	200	60	—
Coccolithus pelagicus	—	—	—	—	—	—	—	—	40
Pontosphæra Huxleyi	146,000	105,400	198,000	308,000	130,200	13,200	9500	6460	1880
Eutreptia viridus	—	—	—	200	—	—	—	—	—
Ceratium longipes	100	—	—	—	—	—	—	—	—
Exuviælla baltica	—	400	—	100	—	—	—	—	—
Gymnodinium Lohmanni	—	—	200	200	200	—	100	80	—
" sp.	600	400	700	300	100	—	100	160	200
Minuscula bipes	—	—	—	100	—	—	—	20	—
Oxytoxum gracile	—	—	100	—	—	—	—	—	—
Peridinium sp.	—	200	—	—	—	—	—	20	—
Acanthostomella norvegica	—	—	—	—	200	—	—	20	—
Laboea conica	—	—	—	100	—	—	—	20	—
" emergens	—	100	—	—	100	—	—	20	—
" reticulata	—	—	500	600	100	—	—	—	—
" strobila	—	—	—	100	—	—	—	—	—
Lohmanniella oviformis	200	300	—	800	2300	100	—	40	—
" spiralis	—	—	300	200	—	—	—	120	60
Mesodinium rubrum	—	—	100	—	—	—	—	—	—
Bodo marina?	6700	3800	9500	5300	5500	300	500	80	20
Forms not identified	1100	300	900	1900	3800	100	300	220	100

TABLE II

Station 1723, July 18, 1933. Latitude, 41°19′ N, Longitude, 66°40′ W.

Depth in meters	1	5	10	15	25	30	40	50	60
Temperature, ° C	15.45	15.47	15.39	13.92	8.67	8.47	8.44	8.21	8.07
Salinity, ‰	33.12	33.15	33.54	33.27	32.51	32.53	32.56	32.56	32.60
Oxygen, cc. per liter	6.18	6.44	6.30	6.58	6.40	6.38	6.24	6.23	6.33
Oxygen, % saturation	107	112	110	111	97	96	94	93	94
PO_4, mg. per cu.m	28	31	28	49	65	68	76	72	75
NO_3, mg. per cu.m	14	11	8	9	53	54	67	62	62
Number cc. examined	25	10	10	10	10(25)	10	10	10	10
Phytoplankton, cells per liter:									
Chætoceros decipiens	—	—	—	—	—	900	500	—	—
Corethron hystrix	40	—	100	—	—	—	—	—	—
Coscinosira œstrupi	—	—	—	—	—	100	—	100	500
Detonula confervacea	400	600	600	1300	200	—	—	100	—
Guinardia flaccida	—	100	—	700	1800	5300	3700	3900	2200
Leptocylindrus danicus	—	—	—	200	800	—	—	—	—
Melosira sp	—	—	—	—	—	100	—	—	—
" sulcata	—	—	—	—	—	—	400	—	—
Nitzschia closterium	40	—	—	—	—	—	100	100	100
" delicatissima	320	—	500	100	200	—	—	400	300
" seriata	—	—	—	—	—	200	—	—	—
Rhizosolenia alata	120	300	—	—	—	—	—	—	—
" fragilissima	40	—	—	—	—	100	—	—	—
" semispina	—	—	100	—	—	—	—	—	—
" styliformis	—	—	—	350	200	1500	—	800	50
Thalassiosira decipiens	—	—	—	—	—	200	—	—	—
" gravida	—	—	—	—	—	—	—	—	100
" sp.	—	—	200	—	—	—	—	—	—
Coccolithus pelagicus	—	—	—	—	200	—	—	—	—
Pontosphæra Huxleyi	67,490	50,500	64,200	120,000	30,000	9,100	1,800	2,300	1,400
Syracosphæra dentata	80	100	—	600	—	—	—	—	—
Eutreptia viridis	—	—	100	6500	600	—	—	—	—

TABLE II—*Continued*

Depth in meters	1	5	10	15	25	30	40	50	60
Temperature, ° C.	15.45	15.47	15.39	13.92	8.67	8.47	8.44	8.21	8.07
Salinity, ‰	33.12	33.15	33.54	33.27	32.51	32.53	32.56	32.56	32.60
Oxygen, cc. per liter	6.18	6.44	6.30	6.58	6.40	6.38	6.24	6.23	6.33
Oxygen, % saturation	107	112	110	111	97	96	94	93	94
PO_4''', mg. per cu. m.	28	31	28	49	65	68	76	72	75
NO_3', mg. per cu.m.	14	11	8	9	53	54	67	62	62
Number cc. examined	25	10	10	10	10(25)	10	10	10	10
Ceratium longipes	—	—	—	—	20	—	—	—	—
" tripos	80	—	—	—	—	—	—	—	—
Exuviælla baltica	120	300	200	—	—	—	—	—	—
Gonyaulax Tamarensis	—	100	—	—	—	—	—	—	—
Gymnodinium Lohmanni	480	300	400	200	100	—	—	—	—
" sp.	320	700	100	1500	300	—	—	—	100
Minuscula bipes	120	—	—	—	—	100	—	—	—
Oxytoxum gracile	440	200	200	800	—	—	—	—	—
Peridinium triquetrum	—	—	—	100	—	—	—	—	—
" trochoideum	—	—	100	—	—	—	—	—	—
Acanthostomella norvegica	—	—	—	100	—	—	—	—	—
Labœa conica	80	—	100	—	—	—	—	—	—
" emergens	—	—	100	100	—	100	—	—	—
" reticulata	80	200	—	—	—	—	—	—	—
" strobila	400	400	—	—	—	100	—	—	—
Lohmanniella oviformis	—	—	—	200	100	200	—	—	—
" spiralis	—	—	—	—	—	—	—	—	—
Mesodinium rubrum	360	200	100	—	—	—	—	—	—
Woodania conicoides	120	—	—	—	—	—	—	—	—
Bodo marina?	520	1800	700	1600	100	800	400	100	400
Forms not identified	640	1000	1500	2000	—	600	600	100	200

atoms and *Ceratia* were the predominant forms and *Pontosphæra* was recorded only in comparatively small numbers; while in 1933 there was a predominant *Pontosphæra* vegetation. (The temperature of the surface layers was as high in 1932 as in 1933 and the salinity even higher.)

Gran (1933) recorded a poorer vegetation in the eastern part of the Gulf than further west. Since the observations were not taken in precisely the same locality, local differences in the plankton distribution may account for a part of the differences which have been recorded in the plankton of the two years; but we should hold it likely that yearly

TABLE III

Comparison of observations at stations 1722 and 1723

Station	Depth	Temp.	Salinity	O_2	Guinardia	Detonula	Pontosphæra
	meters	° *C.*	‰	*% sat.*	*cells/l.*	*cells/l.*	*cells/l.*
	1	13.17	32.07	107	—	200	146,000
	6	13.04	32.07	105	—	100	105,400
	12	12.52	32.09	108	—	—	198,000
	18	11.39	32.11	109	—	200	308,000
1722	30	10.46	32.19	107	—	100	130,200
	42	8.50	32.42	99	—	—	13,200
	54	7.01	32.63	94	—	200	9,500
	66	6.59	32.75	92	—	60	6,460
	78	5.77	32.80	88	—	—	1,880
	1	15.45	33.12	107	—	400	67,400
	5	15.47	33.15	112	100	600	50,500
	10	15.39	33.54	110	—	600	64,200
	15	13.92	33.27	111	700	1300	120,000
1723	25	8.67	32.51	97	1800	200	30,000
	30	8.47	32.53	96	5300	—	9,100
	40	8.44	32.56	94	3700	—	1,800
	50	8.21	32.56	93	3900	100	2,300
	60	8.07	32.60	94	2200	—	1,400
	70	7.98	32.60	94	3600	—	2,800

fluctuations in the composition of the phytoplankton are still more important in producing the state of affairs which has been observed.

For Georges Bank there is a similar difference in the quality of the phytoplankton. In 1932 there was observed here a rich *Rhizosolenia* vegetation with *Rh. alata* as the dominant species. This was then recorded in great numbers throughout the water column, with a maximum of 50,000 cells per liter at 25 meters. This species was also recorded in fairly large numbers from all the stations over the Bank, although it was not so abundant at the two outer stations. In 1933 at Station 1723 *Rhizosolenia alata* was recorded with a maximum number of

only 300 cells per liter. One might seek the explanation for the variation in the occurrence of this species in the fact that this station was located a little further out than the stations of the previous year. However, the abundance of *Guinardia flaccida* at Station 1723 seems to be a good indication that the station was located within the Bank area. The previous year *Guinardia* was also found in the deeper samples from all the stations over the Bank, while it was not observed in the samples from the deeper part of the Gulf (Gran, 1933, p. 177).

These few observations suggest that there exist considerable fluctuations in the composition of the phytoplankton of the waters of the Gulf of Maine and adjacent areas in the summers of different years, perhaps not so much in a purely qualitative respect as in the relative abundance of the various species. The observations on net haul material have given similar indications for the larger forms (Bigelow, 1926). These variations certainly are tied up with changes in the hydrographical conditions from one year to another. For example, the occurrence of *Guinardia flaccida* solely on the Bank (and only in the deeper layers) suggests dependence upon fairly turbulent waters. The question of the relationship between the phytoplankton production and the hydrographical conditions is, however, so complex that we shall not consider it further, since the data for only two stations would not suffice as a basis for discussion. At least two years of intensive simultaneous investigation of the hydrography, the plankton, and the various environmental factors would seem necessary in a study of the influence of the water movements on the supply and conditions of growth of algæ and an explanation of yearly fluctuations. A consideration of the relationship between phytoplankton production and hydrography, especially in regard to the seasonal changes in the phytoplankton occurring in the Gulf of Maine and the Bay of Fundy will appear shortly (Gran and Braarud, 1934).

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GERM CELLS AND SEX DIFFERENTIATION IN LEBISTES RETICULATUS

H. B. GOODRICH, J. E. DEE, C. M. FLYNN,[1] AND ROWENA N. MERCER

WESLEYAN UNIVERSITY

In this paper there is presented a condensed account of the results of a series of studies begun in our laboratory three years ago which deals chiefly with the history of the germ cells in *Lebistes reticulatus* and the correlation of that history with the secondary sex characters. In so far as the results closely parallel those obtained on other fish, the account will be found to be much abbreviated. A brief summary of literature dealing with *Lebistes* is also included.

SUMMARY OF LITERATURE OF LEBISTES RETICULATUS

Lebistes reticulatus, known to tropical fish fanciers as the "guppy," is proving to be valuable material for various types of biological investigations. This species is a native of Venezuela, Trinidad, the Barbadoes, and Leeward Islands. It has been introduced into various tropical and subtropical countries as a means of mosquito control. It seems to have been first classified as *Pœcilia reticulatus* by Peters (1859), and Fillippi (1861) first included it in the genus *Lebistes.* Since then it has been assigned to various genera (*Girardinus, Acanthophacelus*) and is now known as *Lebistes reticulatus* (Regan, 1913). Regan classified it in family 294, the Pœciliidæ, and Jordan (1923) placed it in family 290, the Cyprinodontes. It seems to have made its first appearance as an aquarium pet in Germany in about 1905 (Wichand, 1906).

The brilliant color variations of the male have made it suitable for genetic investigations and the marked sex dimorphism has suggested its use for problems concerned with the determination and physiology of sex. The genetic studies were initiated by Schmidt (1919, 1920) and continued in a series of papers by Winge (1922*a*, 1922*b*, 1923, 1927, 1930, 1932) and by Blacher (1926*a*, 1926*b*, 1927, 1928) and by Eloff (1932).

These studies established the existence of a large number of genes carried by the Y chromosome, a few limited to the X chromosome, some which showed crossing over between X and Y, and one which is auto-

[1] All work on postnatal stages and correlation with secondary sex characters has been done by C. M. Flynn.

somal. A brief review of the earlier papers is given by Goodrich (1929). On account of the unusual situation of the great preponderance of sex-linked genes, Demerec (1928) suggested that the genes might be carried in a chromosome complex rather than in a single Y chromosome, but there has so far been found no cytological confirmation of this suggestion. Independent studies on the sex ratios by Huxley (1920) had led to the deduction that the female was homogametic and the male heterogametic which was, as seen above, confirmed by genetic analysis.

Winge (1927, 1932) considered that his genetic results pointed to the conclusion that in *Lebistes* the male sex is determined by a single dominant gene carried by the Y chromosome and absent in the X chromosome. This theory, however, has been criticized (see Witschi, 1932, p. 206).

The cytology of *Lebistes* has been investigated by Winge (1922*a*), Vaupel (1929), and Iriki (1932), who find a haploid count of 23 chromosomes.

Breder and Coates (1932) have made an interesting investigation of the sex ratios of *Lebistes* when confined in aquaria and include precise data in regard to various details of the life cycle. Chambers and Harvey (1931) have used *Lebistes* as material for the study of ultrasonic waves.

Ginsburg (1929) gives an account of the development of the color pattern. We know of no other work on the development of *Lebistes* except the abstract of a paper read by title only (Dildine, 1933). As far as we can judge, our paper is concerned chiefly with stages earlier and later than those studied by Dildine but otherwise confirms his results.

Lebistes reticulatus is one of the easiest of tropical fishes to rear and methods of handling it are now known to all fish fanciers. Excellent accounts of methods have been given by Winge (1927) and Eloff (1932).

Methods

Since *Lebistes* is viviparous, the embryonic stages are obtained by removal from the body of the female. Females were first selected at random from a mass culture, but in obtaining early stages it was found more practical to isolate individual fish and open each at known intervals after they had given birth to young. Because a single mating may provide sperm for four or five litters (Breder and Coates, 1932), it is not usually necessary to wait for a second pairing. Even under these conditions there is considerable variation in the age of embryos. Bouin's fluid was the principal fixative used except for very early stages when it

was not practical to remove the embryo from the yolk. Under these conditions variations of Slifer's modification of Petrunkevitch's method (Slifer, 1933) were employed. Embryos were passed rapidly through the alcohols and cedar oil was used as a clearing agent. Staining was done chiefly with Heidenhain's iron hematoxylin and eosin. The very early stages were first totally stained in Delafield's hematoxylin for purposes of orientation and later destained. Altogether, sections suitable for study of about 100 eggs and embryos have been obtained. Measurements of early stages are from fixed eggs and those of later stages from living embryos. The measurements of later stages have been made from tip of head to base of caudal fin.

TABLE I

Counts of Germ Cells

Embryo	Length	Number of germ cells	Embryo	Length	Number of germ cells
	mm.			*mm.*	
1	.5	40	15	3.2	61
2	.6	52	16	3.6	192
3	.6	61	17	3.7	107
4	.7	37	18	4.2	223
5	.8	62	19	4.8	305
6	1.0	65	20	4.9	300
7	1.0	54	21	5.2	318
8	1.0	67	22 ♀	5.9	196
9	1.3	64	23 ♂	6.0	393
10	1.5	57	24 ♀	6.3	183
11	1.6	63	25 ♂	6.5	508
12	2.3	66	26 ♀	6.6	627
13	2.5	52	27 ♂	6.7	317
14	3.1	66	28 ♂	7.0	332

THE PRIMORDIAL GERM CELLS

It has been possible to identify the primordial germ cells and to follow their history from the stage of the early embryonic shield until they become finally located in the gonads. They are of large size and of oval shape, averaging about $14\,\mu \times 10\,\mu$, although the greatest dimension may reach $20\,\mu$. The nucleus is large, often bilobed, with a chromatin network less dense than that found in somatic cells. The nucleolus is variable. After fixation in Bouin's fluid and Carnoy Lebrun fluid the nucleus is closely surrounded by a condensation of cytoplasm. Our observations have convinced us of the actuality of this part of the history of the germ cells. The problem of their relation to definitive germ cells is discussed below.

Counts of the number of germ cells present at various stages are shown in Table I. The difficulty of certainly identifying parts of the same cell when divided in sectioning has introduced an error into the counts, especially of the later stages. The earliest counts from the embryonic shield show about 40 germ cells, and there is very little increase until the 3.5-mm. embryo is reached, when there is a period of rapid multiplication followed by a gradual increase through later stages. Sex differentiation of the gonads is recognizable at about 5.5 mm., shortly before hatching. The sex is not, however, indicated by a relative increase in the number of germ cells of the female, as in *Platypœcilus* (Wolf, 1931), but by the increased size of the oögonia.

General Features of Embryonic Development

The egg of *Lebistes* averages about 1.7 mm. in diameter. It is a typical telolecithal teleost egg having meroblastic cleavage. Our sections of the cleavage stages indicate that the early blastodisc has a diameter of about .4 mm., covering about one-thirteenth of a circumference of the egg. Gastrulation involving the formation of the embryonic shield takes place when the blastoderm is about 1.3 mm. in diameter, covering one-fourth of a circumference of the egg. The medullary plate is soon laid down and an embryo of from 8 to 10 somites and of 1 mm. in length is found when the blastoderm has covered only one-half of the yolk. The circulation is established by the time the blastopore closes, when the embryo has 20 somites and is 2 mm. in length. The period of gestation is 28 days at 25° C. (Breder and Coates, 1932) and the young are born when 6.5 mm. in length and with the yolk sac already absorbed.

Explanation of Plate I

Fig. 1. Posterior portion of a sagittal section of an early embryonic shield showing eight germ cells in the mesentoderm. 614 ×.

Fig. 2. A reconstruction of an embryonic shield showing the position of forty germ cells. 61 ×.

Fig. 3. Eight-somite stage showing the number and position of germ cells in the lateral mesoderm. 20 ×.

Fig. 4. Fourteen-somite stage showing the number and position of germ cells in the mesoderm under the somites. 27 ×.

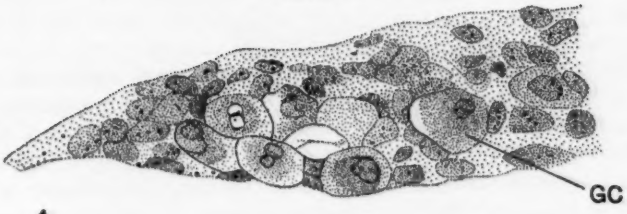
Fig. 5. From a cross-section of a 2-mm. embryo at the time of blastopore closure showing four germ cells ventral to the Wolffian ducts. 614 ×.

Fig. 6. A cross-section of a testis from a 6-mm. embryo showing spermatogonia. 614 ×.

Fig. 7. A cross-section of an ovary from a 6.4-mm. embryo showing oögonia. 614 ×.

Abbreviations: *GC*, germ cell; *Int*, intestine; *Oog*, oögonia; *spg*, spermatogonia; *St*, stroma cells; *WD*, Wolffian ducts.

PLATE I



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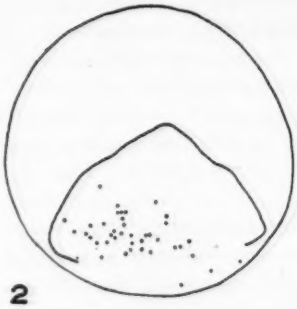

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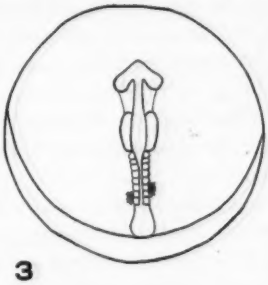

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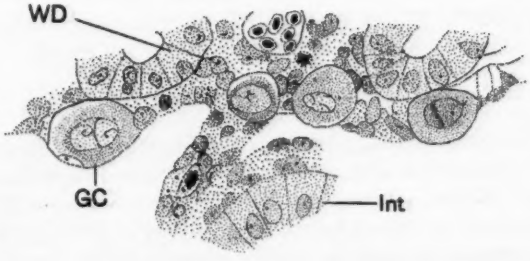

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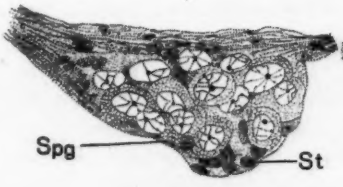

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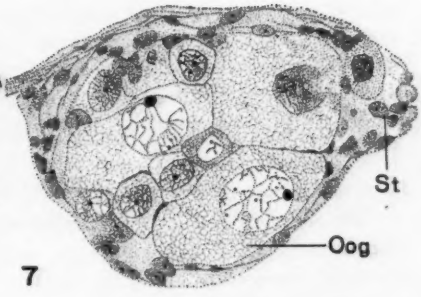

7

Description of Stages in Development

Embryonic Shield

The earliest stage in which it has been possible to distinguish germ cells is that of an early blastoderm (1.3 mm. diameter), which has an embryonic shield measuring .5 mm. along the sagittal axis and .72 mm. in greatest breadth. Before this period the identification of germ cells is uncertain as all cells are nearly of the same size. This stage is represented in Fig. 2, which is a reconstruction based on serial sections. In this are shown the approximate locations of the 40 germ cells which have, according to our criteria, been satisfactorily identified. It will be noted that the germ cells are widely and also asymmetrically distributed. These cells are in the underlying (invaginated) or mesentodermal layer (Fig. 1). Some degree of inequality of distribution of germ cells on either side of the embryonic axis has been observed up to and including the 14-somite stage, after which time they move to a median position.

In a somewhat later stage, in which the medullary plate is clearly defined, but before somite formation, the germ cells were found to be disposed in a U-shaped configuration with the base in the posterior median region and with either arm extending laterally to the medullary plate. It appears that as the medullary plate thickens and forms posteriorly it separates the cluster of germ cells into two groups.

Embryo of 1.26 mm.—Eight Somites (Fig. 3)

As the somites develop, the germ cells are found to be lying in nests in the mesoderm lateral to the somites. In the embryo figured there were about 44 germ cells which were somewhat asymmetrically disposed. Twenty-four were counted on the right side and twenty on the left side and the group on the right side extended further anteriorly than that on the left side. We have no observations which indicate that germ cells migrate through somites, as observed by Dodds (1910) on *Lophius*, but rather they seem to be pushed laterally first by the medullary plate and later by the formation of somites. The situation in this stage is closely similar to that described by Wolf (1931) for a 1.6-mm. embryo of *Platypœcilus*.

Later Stages

The later stages so closely parallel those of *Platypœcilus* that we are omitting detailed description of the individual embryos. Our observations are, however, based on the study of 16 embryos (listed in Table I) of stages from 2.5 mm. to 7 mm. in length.

During this period the germ cells first aggregate (2.7 mm.) medially ventral to the level of the Wolffian ducts and dorsal to the gut. Figure 5 shows a cross-section of an intermediate stage in the migration of germ cells (in an embryo of 2.0 mm.) and Fig. 4 an outline drawing of a 14-somite (1.5-mm.) embryo. Shortly after (3.1 mm.), the median strand of germ cells divides and they enter the newly-forming genital ridges (*cf.* Wolf, 3.5-mm. stage).

The development of the swim bladder now separates this region from that of the Wolffian ducts, which remain dorsal to the swim bladder while the genital ridges are ventral. In a 3.6-mm. embryo the genital ridge has become largely separated from the body wall and may be termed an indifferent gonad.

The period of gonadal differentiation occurs shortly before birth in embryos of about 6 mm. in length. Our few counts of germ cells (Table I) do not indicate, as in *Platypœcilus,* that at this stage there are more germ cells in the female than in the male. The chief criterion is the larger size of the germ cells in the ovary as contrasted with those in the testis. Also the nucleolus of the oögonia is usually eccentric and the chromatin threads stain more heavily than those of the spermatogonia (Figs. 6 and 7).

Female Gonad

The development continues much as described for *Platypœcilus.* The central region of the gonad becomes filled with stroma cells among which the ovarian cavity appears a few days after birth (7-day, 7-mm. fish). The two ovaries gradually approach and fuse and external indications of the double origin disappear at about 35 days after birth. In a 56-day specimen the ovarian cavity is lined with columnar epithelium among which are found oval cells with dense circular nuclei. These are similar to those which Wolf considers to be transitional stages between the epithelial cells and germ cells. There occurs no condition of complete disintegration of primordial germ cells prior to formation of the definitive germ cells such as that described by Essenberg (1923) for *Xiphophorus.*

The Mature Ovary

In the mature ovary the ovarian cavity is central, traversing its entire length, and is continued posteriorly as the oviduct opening into the urogenital sinus. The fully-developed ova extend from the surface to the ovarian cavity and are surrounded by a sheath of follicle cells.

Male Gonad

The spermatogonia do not increase in size as do the oögonia and in the nuclei the nucleolus is usually central and chromatin threads are

fine and lightly staining. At first the germ cells are relatively uniformly distributed in the gonad among the stroma cells but soon (6.9 mm. at birth) the spermatogonia tend to migrate to the periphery. Later (8.2 mm., 21 days after birth) the sperm ducts appear as narrow slits surrounded by the stroma cells. The gonads are also now connected by a bridge of stroma cells. At about 36 days after hatching (9.5 mm.), the primary spermatogonia have begun to divide to form nests of cells which, when surrounded by connective tissue sheaths, form the earliest stages of the typical cysts of the mature testis. At no time have we discovered stages indicating that stroma cells or epithelial cells of the sperm ducts transform into germ cells. In this respect our findings accord with those of Wolf on *Platypœcilus* but contrast sharply with the observations of Essenberg on *Xiphophorus*.

The 48-day stage (12.8 mm.) is of especial interest because this is the period of the first appearance of external secondary sex characteristics. Sections of the testis show a marked increase in the number of cysts. Spermatogonia are in frequent division. Growth stages are abundant and a few spermatocyte divisions have been observed. No mature sperm are present. The sperm ducts are well-developed and cuboidal cells forming their walls are clearly defined. Branches of the ducts extend into the body of the testis.

In a 61-day specimen (14 mm.) spermatocytes and Sertoli cells are present but no spermatozoa have formed.

A 72-day specimen (15 mm.) shows all stages of spermatogenesis and a few of the cysts containing mature sperm have moved into the anterior sperm ducts, there becoming spermatozeugma or spermatophores.

The Mature Testis

The testis is a whitish bilobate body some 2 to 3 mm. in length, located in the dorsal posterior part of the body cavity ventral to the swim bladder and anterior to the cloaca. The two branched internal ducts unite to form a short single duct which opens into the urogenital sinus. These internal ducts are lined by a cuboidal epithelium. We find no evidence that portions of the duct separate from it to form acini as described by Essenberg (1923). Spermatophores are found at various points in the lumen of the sperm ducts.

From the above observations the progress of spermatogenesis may be summarized as follows: The primary spermatogonia at the periphery of the testis by division give rise to nests of secondary spermatogonia which become surrounded by connective tissue sheaths derived from the stroma cells. Formation of new peripheral cysts forces the earlier

ones inward. During this period the various phases of spermatogenesis take place within a given cyst until it becomes filled with mature spermatozoa and the Sertoli cells are located at the periphery. The cyst is then forced into the sperm duct, becoming a spermatophore.

A special search for interstitial cells has been made at the various stages. They are, however, relatively scarce and there does not appear to be any clear correlation between the abundance of interstitial tissue and the appearance of secondary sex characters. Previous work on fish (see review by Rasmussen, 1928) shows that the interstitial tissues apparently take no such important place in the secretion of hormones as appears to be the case in mammals.

Discussion of the Germ Cell Cycle

The problem of the first differentiation of the primordial germ cells in *Lebistes* is perplexing because of the fact that when first recognizable they are widely scattered in the mesentoderm. This distributed condition does not readily fit into theories of embryonic segregation or induction. It may be supposed that there exists an embryonic segregate prior to gastrulation and that the cells then determined have become widely scattered in the spreading of the blastoderm. Even so, however, the determination is most likely to be of the labile or reversible type because, if we accept the results on induction of twins in other fish (*cf.* Stockard, 1921), it appears that any part of the germ ring can give rise to a complete embryo. Under these conditions only those of the potential germ cells which happen to enter the embryonic shield ultimately become the irreversibly determined primordial germ cells.

The later history of the germ cells until the period of formation of the "definitive" germ cells is quite in accord with accounts of the conditions in other fish. In regard to the moot question of the relation of the primordial germ cells to the definitive germ cells, we can only say that our observational evidence indicates clearly that primordial germ cells do give rise to definitive germ cells. The evidence of transformation of other cells (stroma cells) into definitive germ cells is inconclusive. Cells which might be considered transitional have been discovered in the ovary but not in the testis. We realize that this question can only be solved by experimental methods such as those utilized by Heys (1931) and Butcher (1932) which are not readily applicable to our material.

The Secondary Sex Characters

In *Lebistes* the two sexes are externally indistinguishable at birth. At maturity the female is distinctly larger than the male, averaging about

30 mm. in length from tip of snout to the base of the caudal fin, while the male averages 18 mm. in length. The female is of an olive brown color with the edges of the scales somewhat darker, producing a net-like effect—hence the specific name *reticulatus*. Refraction of light from the side of the body may give violet and silvery effects. Fins are colorless except the caudal, which may be yellowish or light green at the basal part.

The male is brilliantly colored, varying according to the genetic constitution (see colored plates, Winge, 1927). In addition to the brilliant

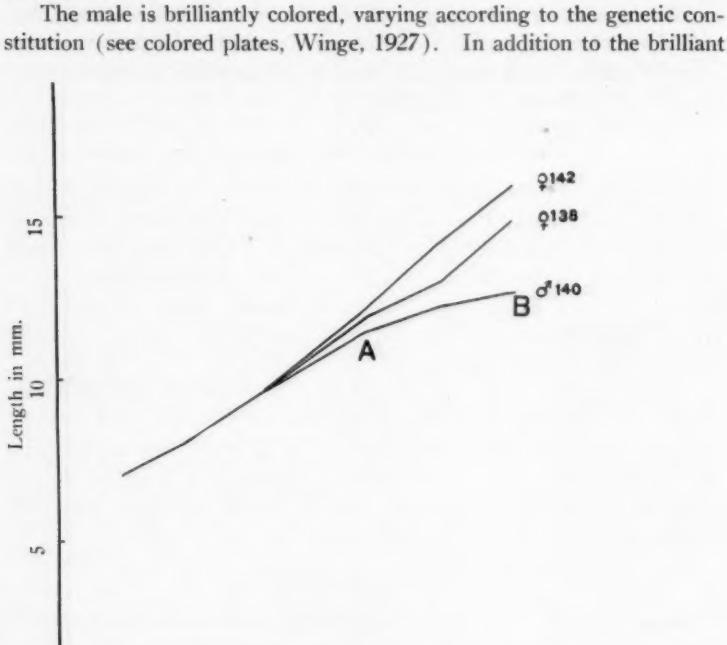

FIG. 1. *A*. Time at which black spots appeared and gonopod became elongated in ♂140. *B*. Time at which colors appeared and gonopod was well developed in ♂140.

spots or areas of red, blue, green, etc., there are a number of paired black body spots in phenotypes of our stock (*cf*. Winge, 1927). These latter are the markings of the male which appear first in development preceding any other recognizable sex character. In the male the anal fin is developed to form an intromittent organ or gonopod.

The size relations and development of black spots were especially followed in the attempt to establish a correlation between the development of the gonads and the secondary sex characteristics.

Fish were anesthetized, examined, and measured at 10-day intervals and from these lots individuals were taken for study of the gonads. All were kept in similar aquaria and under identical temperature conditions. It was usually necessary to keep fish isolated for identification. Isolation, however, appears to retard the growth rate. They thrive better when a number are reared together. If food is discovered by one in a group, the active motions bring other fish to the source of supply; otherwise it often remains undiscovered. Other factors may be involved (*cf.* Allee, 1931). On this account our records may deal with a somewhat retarded development.

A graph (text fig. 1) shows a typical record of three fish. It will be noted that the divergence in size occurred in this case at about 35 days after birth and the black spots on the male and beginning of elongation of the gonopod at 48 days. Sometimes there are size differences at birth, the female being slightly longer, but the growth of all is at nearly the same absolute rate until a point of divergence begins between 30 and 53 days of age. The black spots were first detected at 48 days but sometimes not until the sixtieth day. The elongation of the gonopod was also first noticed at 48 days but does not become marked until about 60 days. Other colors develop more slowly, first appearing at from 60 to 70 days. Full functional maturity of males was attained at from 80 to 100 days, while females bear their first litter between 90 and 120 days. If 28 days is allowed for gestation, this points to a similar or slightly earlier period of maturity than in the male. Inadequate feeding, as observed in the care of some thirty individuals, will markedly retard both the growth and the appearance of the black spots in the male.

If the data in regard to size and other secondary sex characters are compared with that in regard to the development of the gonads, it will be found that in the male there occurs a period of rapid spermatogonial multiplication beginning at about 36 days and continuing until after the 48-day period, when secondary sex characters appear.

At the corresponding period in the female there is no rapid multiplication of oögonia. They are chiefly increasing in size. In neither case is there any marked development of interstitial cells.

These results in regard to the relation between the testis and secondary sex characters are in accord with the general results on fish. Rasmussen (1928) and Moore (1931), who have summarized the evidence, point out that the conditions in fish have not been found uniform. In some fish (Courrier, 1921, 1922) there appears to be an increase of in-

terstitial cells at the breeding season when nuptial colors appear, but in other cases no such correlation has been found. There is some evidence, however, that the presence of the testis as a whole does affect a sex coloration. Kopec (1927) has concluded from his castration experiments that the presence of the testis was necessary for the development of nuptial colors, and Blacher (1926*b*) points out that in cases of sex reversal from male to female in *Lebistes* there is testicular atrophy.

Summary

1. The history of primordial germ cells in *Lebistes reticulatus* has been traced from the early gastrula (embryonic shield) until they become definitive germ cells in the ovary and testis.

2. When first observed, the primordial germ cells are scattered irregularly in the mesentoderm.

3. The formation of the medullary plate separates the germ cells into two lateral groups which become again more widely separated by the formation of somites and lie in the lateral mesoderm.

4. By the 2.7-mm. stage the germ cells are located in a median strand dorsal to the gut. Slightly later (3.1 mm.) they move laterally to the genital ridges.

5. Visible sex differentiation of gonads occurs shortly before birth (6 mm.).

6. The evidence indicates that primordial germ cells give rise to definitive germ cells. There is slight evidence that stroma cells may also give rise in the female to definitive germ cells. No such evidence has been found in the male.

7. Divergence in size between the sexes occurs about 35 days after birth and the first appearance of markings on the male at 48 days. This is coincident with a period of rapid multiplication of spermatogonia and progress of growth stages of spermatogenesis. No correlation with multiplication of interstitial cells has been observed.

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OBSERVATIONS ON REPRODUCTION, PREMATURATION, AND FERTILIZATION IN SABELLARIA VULGARIS

A. J. WATERMAN

(From the Department of Biology, Brooklyn College,[1] and the Marine Biological Laboratory, Woods Hole)

INTRODUCTION

Sabellaria vulgaris (*cf.* Verrill and Smith, 1874) is a polychæte annelid living in sand tubes on dead *Echinarachnius* and other shells, in Bryozoa nodules, and occasionally on stones. The animals occur apparently in considerable numbers in the vicinity of Woods Hole, Mass., and eggs and sperm may be readily obtained throughout the summer months. Shedding takes place immediately following the removal of the animals from their tubes.

Two European species of *Sabellaria* (*alveolata* and *spinulosa*) have been studied by Fauré-Fremiet (1924), Hatt (1931), and Dehorne (1910*a*, *b*, 1911, and 1913). In addition to describing the origin, mechanics of shedding, prematuration, and fertilization of the gametes of *S. alveolata*, Fauré-Fremiet has analyzed the egg chemically and has provoked artificial parthenogenesis by increasing the alkalinity and elevating the temperature of the sea water. Hatt has studied the organization of the egg of the same species by isolation of blastomeres and cutting experiments, while Dehorne has been especially interested in the behavior of the chromosomes during maturation in the egg of *S. spinulosa*.

No reference has been found in the available literature to any study of the American species, *Sabellaria vulgaris*. For this reason it has seemed of interest to follow the early development of this species, from the time of the separation of the ova from the ovaries to the early cleavage stages, with special reference to longevity of the gametes, fertilization reaction, and the relation of the first cleavage plane to the sperm entrance point and to the position of the polar bodies. In many respects the early development of *S. vulgaris* is strikingly similar to that of *S. alveolata* as described by Fauré-Fremiet. This animal was called to my attention by Professor Earl A. Martin.

[1] Contribution No. 9.

Shedding of the Gametes

Figures 1 and 2, Plate I, are photomicrographs showing the position of the gonads and the presence of gametes in the cœlomic cavity. In Fig. 2 the ovary may be seen as a dark cellular mass to which young ova of various stages of development are attached by narrow stalks, and the cœlomic cavity contains a few irregularly shaped detached ova with large germinal vesicles. As the young ova develop they become separated from the ovary and are stored in the cœlomic cavity which they greatly distend. Extrusion occurs through minute pores (one of which is present in the photograph) in the lower lateral wall of the segment (compare *alveolata*).

The irregular shape of the ova (Fig. 4, Plate I) is said to be caused by crowding in the cœlomic cavity, and, with release of pressure following shedding, the spherical form is attained. In addition to this change of shape, the difference in pH of the sea water from that of the cœlomic cavity results in the elevation of the vitelline membrane and the initiation of the prematuration changes. During this sequence of changes the original point of attachment of the ovum to the ovary disappears.

The normal shedding of *Sabellaria vulgaris* is said by Verrill and Smith (1874) to occur during May and June, but this has not as yet been verified. At other times the release of the worm from its tube is the stimulus for shedding to begin and this continues until most of the eggs have been extruded. A quiescent animal which has ceased shedding may be stimulated to renewed activity by a weak chlorotone solution, indicating that muscular contraction plays a part in the extrusion of the gametes. Eggs and sperm were secured up to the latter part of August, at which time the experiments had to be discontinued. Undoubtedly they could have been obtained later. Both eggs and sperm are shed in considerable quantities depending upon age and size of the animals and the number present in the cœlomic cavities. The shedding time varies between five and ten minutes and may last as long as twenty minutes.

The normal shedding time of May and June is followed by a second but shorter period extending from around August first to fifteenth. The number of fertilized eggs secured during the first part of July steadily decreases, until about the second week very few cases of development occur even though the animals shed together. A high percentage of fertilization can be secured, however, by changing the normal pH of the sea water (at Woods Hole around 8.3) to 8.6 according to the method of Lillie (1923). Around the first of August

evidences of normal fertilization begin to increase and this reaches a high percentage (90 per cent or more) during the second and third week, after which the fertilizability of the ova in normal sea water steadily decreases. The change in alkalinity does not appear to affect the fertilized ova adversely provided they are removed to fresh sea water.

In contrast to the above, the natural shedding time of *Sabellaria alveolata* is August 20, after which new oöcytes may be produced to give another shedding time around September 15. Sexual maturity may occur as early as June and eggs may be secured by placing females in sea water at 10° C. All shed at the same time and the ova mature at the same rate. The same holds true for *S. vulgaris* and in this way a considerable number of eggs may be obtained at one time. Labbé (*cf.* Fauré-Fremiet, 1924) has found females of *S. alveolata* full of eggs in April which were shed freely but could not be fertilized in the ordinary way, while LoBianco describes the finding of females filled with eggs in October and states that these, on shedding, could be fertilized.

The Ovum Previous to Fertilization

Immediately after shedding, the ova are of odd, irregular shapes due to pressure in the cœlomic cavity of the female (compare Just, 1912, on *Nereis*), and a conspicuous vitelline membrane is closely pressed to the surface of the egg (compare *S. alveolata*). No micropyle is visible nor any evidence of the point of original attachment of the growing gamete. The germinal vesicle is a large, vesicular, whitish area more or less eccentrically located. As seen under the microscope, the egg is colored a light green due to the presence of a large amount of deutoplasmic substance distributed throughout the cytoplasm. There is no conspicuous colored granulation and the egg is too opaque for observation of internal processes except in the behavior of the germinal vesicle.

Visible polarity appears at once in a series of prematuration changes involving (1) the breakdown of the germinal vesicle and the flowing of its contents towards one side of the egg to form a clear hyaline cap-like area at the future animal pole, (2) a change in shape of the egg so that it takes on a spherical form, and (3) the elevation of the vitelline membrane (compare *Cerebratulus*). The vitelline membrane rises from the egg surface during and subsequent to the assumption of the spherical form. While no accurate measurements have been made, it appears that the accumulated fluid between membrane and egg is not derived from the egg cortex (*Nereis*), as no conspicuous decrease in diameter of the egg or cortical change is noted, but is the result of

the change in the permeability of the membrane itself. The height of elevation amounts to about one-fourth or even one-third the diameter of the egg. As it rises the membrane is variously folded and these folds may never disappear even in very old eggs or in those that have been fertilized. The membrane may be removed by the intestinal fluids of the blue crab or with strong NaCl solution (*cf.* Hatt, 1932). Prematuration stops at the metaphase stage of the first polar spindle (*cf.* Lillie, 1906).

During its elevation and thereafter fine protoplasmic processes extend from the inner edge of the membrane to the egg surface. These taper towards the membrane and are thicker at the base. Their origin possibly lies in the localized attachment of the membrane to the egg surface and during elevation the cortical hyaline protoplasm is thus drawn out into fine streamers. These strands persist for a long time in unfertilized eggs but shorten and disappear after the spermatozoön has entered.

The elevation of the vitelline membrane, together with the formation of these fine protoplasmic processes previous to fertilization, constitute two phenomena which are not common in other ova. Chambers (1933) describes fine processes, extending between egg cortex and fertilization membrane of the egg of *Echinarachnius* and *Asterias,* which quickly disappear. If the egg of *Cerebratulus* enters the sea water, a vitelline membrane elevates to a considerable distance above the egg cortex and a micropyle (?) is present in the vicinity of the former stalk of attachment. Penetration may occur anywhere and the sperm encounter no difficulty in penetrating the membrane. The changes occurring in the egg of *Nereis* are too well known to require reviewing here. It is sufficient to note that in this case the protoplasmic strands are remnants of cortical material remaining after the extrusion of the jelly and they disappear after fertilization.

If unfertilized eggs are allowed to stand for several hours there frequently occurs a stratification of cytoplasmic components to form three zones, (1) a large dark zone, staining deeply with Nile blue sulphate, which includes oil droplets and yolk material; (2) a smaller granular zone from which the yolk material is absent, and which stains a pale blue; and (3) a clear hyaline zone. These three zones bear no fixed relationship to each other nor to the polar cap of the animal pole, but the hyaline cap formed at the breakdown of the germinal vesicle may lie at one side or form a fourth layer. The cortical clear cytoplasm is more distinct in these eggs. Another result is a lobular fragmentation of egg material that closely resembles cleavage in the earlier stages but soon becomes irregular. This is clearly a degenerative effect and will

be described below in the section dealing with the effects of the change in pH. This fragmentation, occasionally preceded by the appearance of one or two polar bodies, would almost give the impression of artificial parthenogenetic development except that subsequent fragmentation becomes irregular and no swimming embryos develop.

Fertilization

No membrane elevation occurs at fertilization and the only evidence that a spermatozoön has entered, previous to the formation of the polar bodies, is the slow withdrawal and final disappearance of the protoplasmic strands. They persist for hours in unfertilized eggs. As to their function, it is conceivable that they serve to transmit the stimulus of sperm contact to the egg substance across the wide fluid-filled subvitelline space and the one attached to the spermatozoön may draw it into the egg unless it is previously engulfed by the fertilization cone. Their presence may also account for the large size and marked irregularity of the fertilization cone, since cone formation progresses along pathways previously indicated by these strands. During penetration the strands pull away from the inner edge of the vitelline membrane, shorten and thicken and frequently curl or form minute knobs at the free ends. They disappear completely by the time the first polar body forms (Figs. 5 and 6, Plate I).

Penetration may take place at any point around the periphery of the egg but this occurs more frequently in the animal hemisphere. Not every spermatozoön which touches the membrane enters the egg. They may adhere quietly to the membrane for a few seconds and then slip away to become attached to a neighboring egg, while others will adhere quietly for long intervals even after fertilization has occurred. The tails may remain motionless or very slowly whip back and forth causing the egg to vibrate slightly. Approximately thirty minutes after being introduced into the vicinity of the ova, all activity of the spermatozoa ceases and they either are attached to the egg membranes or lie motionless on the surface of the glass slide. Newly-shed eggs fail to produce any renewed activity.

The response of the egg to the entering spermatozoön is the abrupt formation of one or several hyaline exudations which fuse to form a large protoplasmic cone. Several of these cones may be initiated in a single egg but the one which reaches the membrane first is the successful one. This contact overcomes the tendency for accessory cone formation and the others are withdrawn. At first the fertilization cone contains only hyaline-cortical material, but as it enlarges its center becomes

filled with endoplasmic granules. The formation of the cone thus appears as a rupturing effect caused by a lowering of surface viscosity comparable in a sense to the formation of pseudopods. The flowing of the granules into the cone may continue until the cone reaches the membrane. As the cone is withdrawn the granules slowly disappear into the egg, leaving eventually only hyaline material (text fig. 1).

During its existence the huge irregularly-shaped cone is constantly changing in shape, and during withdrawal and after the disappearance of the granules may break up into several bodies one of which may remain in contact with the sperm head. Temporary exudations from the cone itself form, become globular, and may be withdrawn as the cone grows towards the membrane. Actual penetration often involves a considerable interval, several minutes elapsing between the time the cone reaches the membrane and its final disappearance. As the cone is retracted the region of the membrane to which the spermatozoön is attached is pulled inward to form a narrow cavity containing it. Subsequently the sperm head is pulled through the membrane and in this process the head is markedly elongated. When the spermatozoön is about halfway through the membrane, the latter flips away and outward, with the tail or at least a part of it still attached. The membrane was thus under considerable tension. This shows that there had occurred a definite adhesion between membrane and cone which was sufficiently strong to enable the cone to draw the membrane inward against pressure.

In very few cases has the cone been observed actually to engulf the spermatozoön (text fig. 1). In general the latter is attached to the surface of the cone and is thus pulled into the egg (text fig. 2). Fre-

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EXPLANATION OF PLATE I

1. Cross-section of a male, showing a large mass of spermatozoa in the cœlomic cavity. At the right is a small portion of the digestive tube. × 120.

2. Cross-section of a female. In the cœlomic cavity may be seen the darker ovarian mass together with detached ova. To the left is a portion of a nephridial tube containing a compact mass of ova. The animal was fixed immediately after shedding had ceased. × 120.

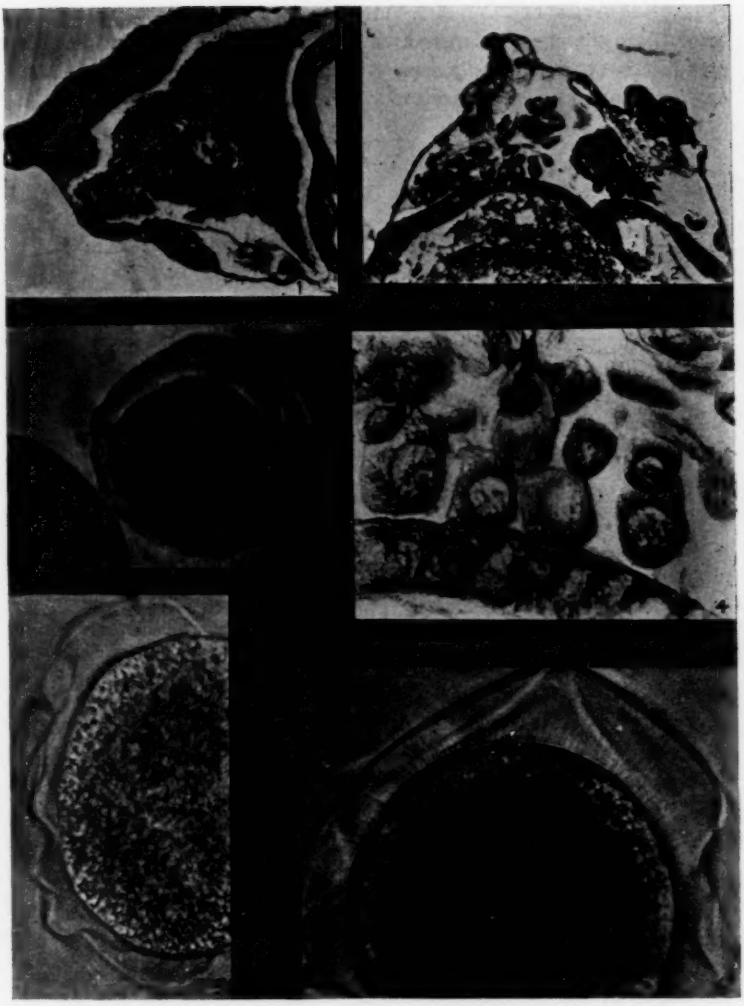
3. A living egg in sea water. The egg has become approximately spherical and the vitelline membrane elevated. × 900.

4. A portion of Fig. 2 under higher magnification to show the irregular shape and large germinal vesicle of the ovum previous to shedding. The vitelline membrane is closely pressed to the egg surface. × 550.

5 and 6. Enlarged photomicrographs of living ova taken before and after the entrance of the spermatozöon. In Fig. 6 the delicate protoplasmic strands, extending between egg cortex and the elevated vitelline membrane, are visible. In Fig. 5 the strands have disappeared, indicating that a spermatozoön has entered.

PLATE I

(All figures are photomicrographs)



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quently just before its disappearance it may be engulfed or the cone may disappear entirely, leaving a thickened thread between membrane and egg surface, which draws the spermatozoön into the egg (text fig. 3). This reaction is comparable to that described by Chambers for the eggs of *Asterias* and *Nereis*. After the penetration of the spermatozoön into the egg the larger area of clear hyaline protoplasm in this area disappears and nothing remains to show where it has entered except external markers such as accessory spermatozoa or folds in the membrane.

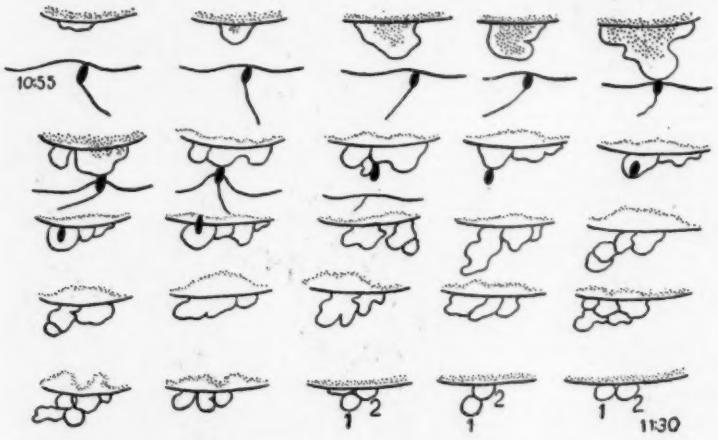

FIG. 1. A series of diagrammatic drawings illustrating the entrance of a spermatozoön into an egg of *Sabellaria vulgaris*. The time interval is indicated. After the large fertilization cone, containing endoplasmic granules, had come in contact with the vitelline membrane at the point of sperm entrance and had pulled the spermatozoön through the membrane, it broke up into several exudations, one of which engulfed the spermatozoön. The flipping out of the membrane following passage of the spermatozoön through it is shown. Following the disappearance of the spermatozoön into the egg, several temporary exudations formed in this region and by 11 : 30 two polar bodies had appeared.

In the case illustrated the thickened thread was the remnant of the fertilization cone from which the more fluid material had been withdrawn. As such it consisted of cortical material only and its withdrawal was but a final step in cone retraction. This phenomenon was seen so many times as to appear significant as a possible explanation of the origin of the fertilization thread described so frequently in the literature.

There are at least three times in the early development as far as the

two-cell stage, in which a conspicuous alteration in the state of the cortical layer of the egg takes place, *i.e.*, at fertilization (cone formation and withdrawal of the protoplasmic strands) and at first and second polar body formation. According to Whitaker (1931), there occurs an alteration of the physical state of the cortical layer at the time of cone formation and its disappearance is marked by an increased viscosity of this layer. A similar change must occcur at the time of polar body formation, in addition to the division phenomenon, since not only is the

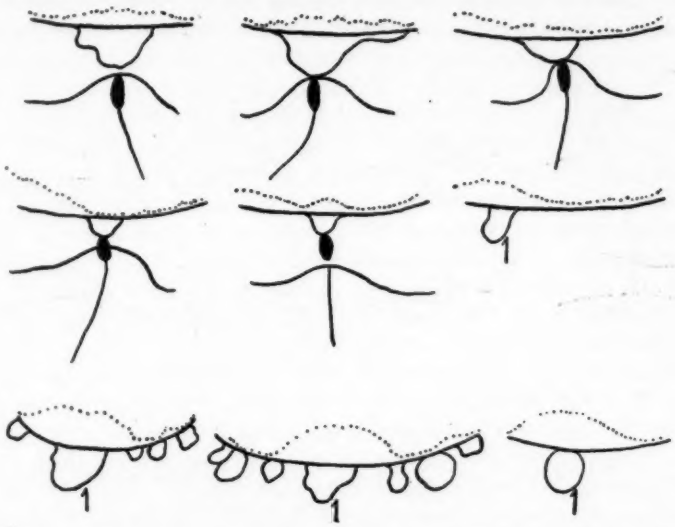

FIG. 2. Another case of fertilization in which the spermatozoön was not engulfed by the fertilization cone which was small and non-granular. The former was drawn into contact with the egg surface by the retraction of the cone. During the formation of the first polar body many hyaline exudations formed but were only temporary.

surface of the egg slightly indented after the polar body is pinched off but hyaline exudations accompany this process. The reaction is not confined locally but extends unevenly outward through the egg surface to a diminishing degree. The more peripheral exudations are generally smaller than those nearer the fertilization cone or polar bodies; they seldom form around the entire egg surface; and moreover they are scattered. The cortical change is not uniform.

In one case the spermatozoön was seen to enter the egg at one side of the polar hyaline area in the vicinity of the maturation spindle.

Considerable activity occurred in this region as evidenced by a bubbling of the cortical area without entire disappearance of the parts of the fertilization cone. All but one of the exudations soon disappeared and this one turned out to be the first polar body (text fig. 1).

The time involved in the early developmental stages has been carefully followed in several cases. Eggs shed at 11:49 were fertilized at 11:49 by sperm shed at 9:00. The fertilization cone reached its maximum size by 11:52, after which it was retracted, pulling membrane and attached spermatozoön with it. By 11:56 it had disappeared, leaving a fine filament extending from egg surface to membrane at the

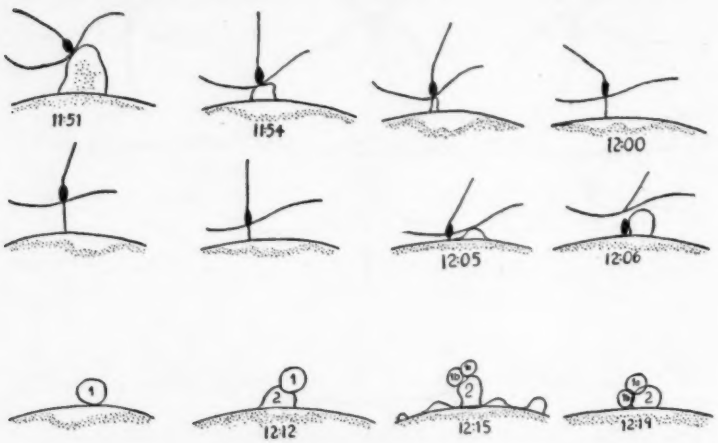

FIG. 3. In this case, the large fertilization cone disappeared, leaving a thickened thread connecting spermatozoön and egg cortex. The spermatozoön was drawn into the egg by the contraction of this thread, but before its disappearance an exudation appeared at its side. This was the first polar body which later divided into two parts. At the time of appearance of the second polar body, several hyaline exudations temporarily appeared in the vicinity.

point of sperm attachment. At 11:59 the other protoplasmic processes had almost disappeared. The filament continued to shorten, pulling membrane and sperm nearer to egg surface, and at 12:05 a second exudation which later turned out to be a polar body appeared at one side of the filament (text fig. 3). The spermatozoön began to penetrate the vitelline membrane at 12:04 and was through at 12:06, the membrane flipping outward as soon as the spermatozoön was through. At 12:06 it was attached to the egg surface in direct contact with the polar body. One minute later it had disappeared into the underlying

protoplasmic cap. The second polar body appeared at 12: 12 and by 12: 15 the first polar body had divided. The polar lobe appeared at 12: 30 and at 1 o'clock 2–4 cell stages were found. This case illustrates the relative instability of the pre-maturation figure since the first polar body appeared before the spermatozoön had actually entered. Several cases of this were observed in which sperm entrance occurred in the region of the clear protoplasmic cap of pre-maturation. During the extrusion of the second polar body several exudations formed in the vicinity but the egg had resumed its spherical shape by 12: 19.

In another case eggs shed at 9: 00 were fertilized at 9: 14 with fresh dry sperm suspension. In all the studies the cover-slip was supported by pieces of No. 1 cover-slip to prevent pressure on the eggs. In this example the sperm entered before pre-maturation was completed. By 9: 25 all the protoplasmic processes had disappeared. The point of sperm entrance was marked by the presence of accessory sperm and a fold in the vitelline membrane. Meanwhile the contents of the germinal vesicle were seen to be moving towards the egg cortex along a path at right angles to the plane of sperm entrance. By 9: 37 several exudations, which lasted for one minute, appeared in the region of the clear polar cap. One remained as the first polar body. For a short interval after the polar bodies were fully formed the egg cortex was slightly depressed or indented just beneath the polar body. The second polar body formed just beneath the first and was complete by 9: 48. Exudations from the adjacent cortex again appeared at this time. At 10: 00 the polar lobe made its initial appearance opposite the region of polar body formation. Polar lobe formation is preceded by a distinct flattening of this region. At 10: 05 the polar lobe had reached its maximum proportion and the first cleavage furrow was indicated by a constriction to one side of the polar bodies. In this example the sperm entrance point bore no relation to the plane of first cleavage but there was slightly less than a 45° deviation. The first cleavage was completed by 10: 13.

The Relation of the First Cleavage Plane to Point of Sperm Entry and Position of Polar Bodies

In the above case the cleavage plane cut the egg in such a fashion that the polar bodies lay on the smaller blastomere and the larger blastomere contained the contents of the polar lobe. Two other developing eggs were in the field at the same time and in both cases a similar relationship was observed. In each case the first polar body divided to form two smaller ones. Later, however, at the two-cell stage, one or more of the polar bodies may come to lie on the large blastomere. Subse-

quent cleavage is more or less regular but it is not uncommon to find three-cell stages. This particular culture gave 97 per cent swimming embryos. The orientation of the first cleavage plane with reference to the positions of the polar bodies has been observed many times.

The entrance point of the spermatozoön appears to bear no fixed relation to the first cleavage plane (text fig. 4). Out of twenty cases only six showed an exact coincidence. In one case in which a spermatozoön entered the polar cap to one side of the median point, the polar bodies were pinched off beside the entrance point and the first cleavage

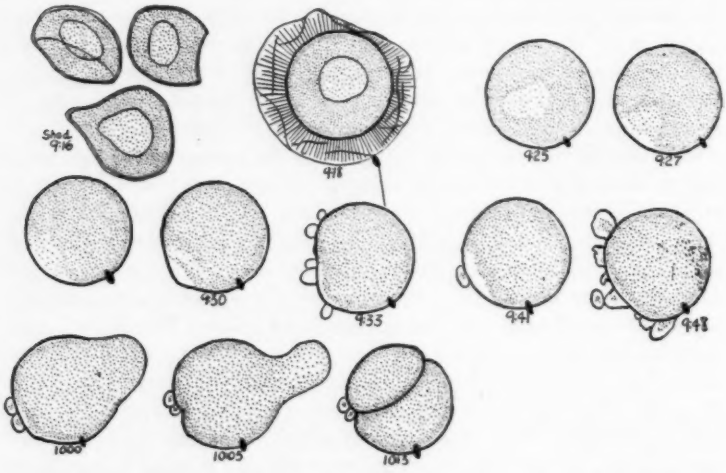

FIG. 4. A series of diagrammatic drawings made from a living ovum to illustrate the early development as far as the two-cell stage. When the eggs are shed they are irregular in shape and have large germinal vesicles. Subsequently they become spherical, and the vitelline membrane elevates. Fine protoplasmic strands which extend across the sub-vitelline space disappear after fertilization. The contents of the germinal vesicle form a clear cap at the animal pole. The plane of first cleavage bears no fixed relationship to the entrance point of the spermatozoön (black body) and passes to one side of the pole as indicated by the position of the polar bodies.

plane passed through this point. While these observations are perhaps indicative, the number of cases followed are as yet too few for any definite conclusion. Morgan and Tyler (1930) found that in *Cumingia* the coincidence occurred in 77 cases while in 21 cases there were less than 90° deviation. In *Chætopterus* 48 cases of coincidence were observed; 35 cases deviated less than 45° and 33 cases more than 45° but less than 90°. In *Nereis* 51 per cent agreement was seen. Wilson and

Matthews observed in *Toxopneustes* that the great majority of first cleavages passed at least approximately through the entrance point. Just (1912) found an even higher percentage of coincidence in *Nereis*. He states that the sperm entrance point determines the first cleavage plane which passes through this point. The first cleavage plane in *Sabellaria* does not pass through the pole as determined by the position of the attached polar bodies, but slightly to one side.

Length of Fertilizing Power of Eggs and Sperm

The majority of the observations made upon the process of fertilization in *S. vulgaris* involved the use of slightly alkaline sea water (8.6), as the normal breeding season, which is said by Verrill and Smith to occur during May and June, had passed and a good percentage could not otherwise be secured. What the condition is after the second but shorter shedding time (early August) is not known, as observations had to be discontinued. Alkalinity was produced by using n/4,000 NaOH in sea water. Eggs shed in chlorotone during anesthesia of the female and fertilized in alkaline sea water gave as high as 92 per cent cleavage.

The most numerous cases of successful fertilization are secured when the sperm and eggs are shed simultaneously in the same medium. This applies either to the use of a more alkaline medium or to fresh sea water. According to Fauré-Fremiet, fertilization of eggs that have stood for more than an hour is uncertain. In the present study the interval appears somewhat longer but the length of egg life is not long. In one case eggs shed at 9:00 A.M. were fertilized at 4:45 P.M. with fresh sperm. The next day no embryos had developed, 10 per cent showed lobular fragmentation, some of the remainder showed stratification, and a few were markedly swollen.

The activity of sperm does not last very long. In one case eggs shed in normal sea water at 12:00 noon (August 17) were fertilized immediately by a heavy dry sperm suspension shed at 11:00 A.M. Twenty-five minutes later no moving sperm were present in suspension but either adhered to the vitelline membranes or were non-motile on the surface of the slide. This culture gave almost 100 per cent swimming embryos. In subsequent cultures the activity of the sperm ceased 20–30 minutes after addition to the egg suspension. Freshly shed eggs added to the cultures caused no reactivity of these sperm. Eggs and sperm shed together at 9:40 gave the same high percentage of swimming embryos.

In another case (August 17) eggs shed at 8:50 A.M. were fer-

tilized by sperm shed at 4:55 P.M. (fresh sea water). Polar bodies were slower in forming, but twenty-four hours later the culture showed approximately 98 per cent swimming embryos. During the interval previous to fertilization no cytolysis, stratification, or fragmentation occurred and protoplasmic strands were still present.

A comparison between the length of fertilizing power of the gametes in alkaline sea water and normal sea water shows some variation. Moreover, in the former case fertilization is occurring between gametes shed during a period when fertilization is not normal or occurs in a low percentage of the cases which represent animals coming late to sexual maturity. Furthermore, the results are not at all certain when alkalinity of the medium is increased slightly.

This question must be studied further, but the data available at the present time seem to show that in an alkaline medium the highest percentage of fertilization occurs when males and females shed together. As high as 98 per cent of swimming embryos have been secured in this way. Dry sperm shed an hour before addition to egg suspension fail entirely to penetrate the eggs. Between these two extremes the percentage of successful penetration decreases. Eggs one to three hours old will give around 96 per cent swimming embryos when fertilized with fresh sperm.

In normal sea water, as observed during the second breeding interval (August 15–22), the length of fertilizing capacity of the gametes is markedly increased. It has been found in some cases that eggs eight hours old gave 98 per cent swimming embryos when fertilized with fresh sperm. Fresh eggs plus sperm one hour old gave only 45 per cent success. The percentage markedly decreased with the age of eggs and sperm. Successful insemination is seldom secured to any extent with sperm more than one hour old. In general it may be said that the highest percentage of fertilization may be expected if the two sexes shed together, whether in sea water or slightly alkaline sea water—a conclusion which agrees with that of Fauré-Fremiet.

Effect of Change in pH on Egg

According to Fauré-Fremiet, the pre-maturation phenomena of the sabellarian egg is initiated by the change in alkalinity of the sea water as contrasted with that of the cœlomic cavity, and it is possible to secure artificial parthenogenesis by raising the pH to 12 or by elevating the temperature to 28° C. In the present observations it has been found that a slight elevation of pH from 8.3 of normal sea water to 8.6 will enable fertilization to occur where normally it does not to any extent;

i.e., from early in July to around the first of August. This increase in alkalinity will not alone induce development nor does it appear to harm the eggs provided they are removed to fresh sea water after the spermatozoa have entered. In some instances, when the fertilized eggs remained in the solution, the polar body formation and the first few cleavages occurred in a typical fashion. Subsequently, however, the cleavage became irregular, some eggs which had formed polar bodies failed to develop further, and no embryos formed. However, other experiments gave a high percentage of swimming embryos.

A few experiments were made involving the effect upon the unfertilized egg of changes in pH. Eggs of the same female were used throughout the series. In some instances the female was allowed to shed for a certain time in one solution and then removed, quickly dried with blotting paper, and transferred to the next solution; this continuing until all the solutions contained eggs. The pH of the various solutions was tested by B. D. H. Universal Indicators and as such are only approximate.

Series A. After one-half hour at a pH of 11, some eggs showed shortened processes or total absence of these photoplasmic strands. Pre-maturation and membrane elevation were typical. After one and one-half hours the eggs were transferred to normal sea water. One hour later out of 55 eggs, 14 showed polar body formation and 2 were at the two-cell stage. Four and one-half hours later 96 per cent showed abnormal cleavage or balls of cells. Twenty-four hours later no embryos had formed and disintegration was far advanced. At a pH of 10.5 fewer showed polar body formation and abnormal cleavage. At a pH of 10 much the same result was seen. With pH of 9.5, 9, 8.6, 8.3 fewer showed polar body formation and abnormal cleavage in the given time. But twenty-four hours later all had fragmented. No embryos developed in any case and disintegrative effects were conspicuous. In the control culture 97 per cent cleaved normally and formed embryos. The latter were eggs which had been fertilized in slightly alkaline sea water (8.6) and then transferred immediately to normal sea water.

This experiment was repeated several times with variations in the length of exposure. In every case the final result was the same (*i.e.*, abnormal cleavage followed by death), although in the solutions with the greater alkalinity the number of eggs showing preliminary polar body formation was slightly higher. Frequently the polar bodies were of varying sizes and occasionally fragmented.

As these preliminary results indicate, no cases of true activation were secured which formed embryos. The initial stages such as retraction and disappearance of the protoplasmic strands and the formation of

the polar bodies compared closely with similar phenomena in fertilized eggs although somewhat delayed. It would appear from this that a change of pH is capable of initiating profound change in the egg immediately after shedding but that at the same time the exposure leads to death.

The instability of the pre-maturation spindle is further shown by the effect of vigorous shaking in a test tube. Unfertilized eggs shaken from one to five minutes behaved in a way comparable to the above; *i.e.,* one or two polar bodies formed, the first few cleavages were frequently more or less regular, but no swimming embryos developed. The earlier cleavages soon became irregular, giving an effect of degenerative fragmentation, since on sectioning many of the cytoplasmic fragments were without nuclear material. Unfertilized eggs from the same original batch left in sea water underwent fragmentation and in many cases the egg contents were stratified into three zones.

Eighty per cent hypotonic sea water failed to cause polar body formation. Eggs were put into this solution forty-five minutes after shedding and were removed to fresh sea water at one-minute intervals thereafter up to fifteen minutes. Other dilutions of sea water gave a similar result.

The observations reported above are of a preliminary type intended primarily to determine the availability of the egg for experimental work. As such they would seem to show that the egg of *Sabellaria vulgaris* offers possibilities for work upon a species that has not as yet been studied to the extent of others common to Woods Hole.

Summary

1. *Sabellaria vulgaris,* a polychæte annelid inhabiting sand tubes on dead *Echinarachnius* and other shells in Bryozoa nodules, and on stones, occurs abundantly in the vicinity of Woods Hole, Mass., and gametes may be secured throughout the summer months. Shedding takes place immediately following the removal of the animals from their tubes.

2. The early development is described from shedding to early cleavage and a comparison made with *Sabellaria alveolata* as described by Fauré-Fremiet. In most respects the early developmental phenomena of these two species are strikingly similar.

3. After shedding the eggs become spherical, the vitelline membrane elevates, leaving fine protoplasmic processes between membrane and egg cortex, and the contents of the germinal vesicle form a clear area at the animal pole. Visible polarity appears during pre-maturation which ceases at the metaphase of the first maturation spindle.

4. It is said that fertilization occurs normally during May and June but a second and shorter time has been found during the early part of August. During July a high percentage of fertilization may be secured with slightly alkaline sea water (8.6).

5. The highest percentage of cleavage is obtained when the gametes are shed together. Eggs may be fertilized after seven hours, but the fertilizing capacity of the spermatozoa is considerably shorter (about one hour).

6. The fertilization phenomenon is described. The protoplasmic strands serve to transmit the stimulus of sperm contact. No fertilization membrane appears and the first indication that a spermatozoön has entered is the withdrawal of the processes.

7. The fertilization cone is enormous and changes shape. Spermatozoa may enter at any point on the periphery but more frequently in the animal hemisphere. The polar bodies are large and the first one frequently divides.

8. Various factors such as increase of pH, shaking, and standing are sufficient to disturb the equilibrium of the pre-maturation spindle with the resultant formation of one or two polar bodies. This is followed by lobular fragmentation and no swimming embryos develop. Old unfertilized eggs stratify into three zones.

9. Plane of first cleavage bears no fixed relationship to sperm entrance point and passes to one side of pole as determined by the position of the polar bodies. The polar bodies lie on the smaller blastomere while the larger one contains the polar lobe material.

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CATALASE AND OXIDATIVE PROCESSES IN ANIMAL TISSUES AS POSSIBLE FACTORS IN ADAPTATION

HOYT S. HOPKINS

DEPARTMENT OF PHYSIOLOGY, NEW YORK UNIVERSITY, COLLEGE OF DENTISTRY

In a previous report (1930) upon the respiratory exchange and catalase in tissues of lamellibranchs, the author pointed out a relationship between the habitat distribution of several species and the relative abundance of their muscle catalase. To these have been added new data for the same and for other species on our southern Atlantic coast, making twelve altogether and representing diverse conditions of environment. The above experiments were performed at the U. S. Bureau of Fisheries, Beaufort, North Carolina, during several summer seasons. A second series of determinations was carried out at the Bermuda Biological Station in the summer of 1931, using four other species.

The data from the Carolina and Bermuda experiments, summarized in Tables I and III, are treated separately because the species are distinct and on account of climatic differences in the two regions. For, although the temperature of the water is practically the same in the summer, the annual variation is greater on the Carolina coast than in Bermuda. The fauna in the latter region is sub-tropical in character.

The Bermuda species of bivalve mollusks used in this study differ in certain respects from those on the mainland. They are attached firmly to the substratum by means of a byssus (*Mytilus, Arca*), or with one valve of the shell cemented to rock or piling (*Chama, Spondylus*)—and all are non-burrowing. A majority of the Carolina species burrow in sand or mud. Of the others, *Modiolus* and *Arca ponderosa* have a byssal attachment and live exposed in varying degrees; *Pecten* is free-swimming; *Ostrea* is attached securely to solid objects, freely exposed. In these species the figures for oxygen consumption are higher and more diverse than in the Bermuda forms. Two varieties of *Arca* observed in Bermuda were entirely lacking in the red blood pigment which characterizes members of the genus on our coast.[1] The tropical conditions seem to be conducive to uniformity in the physiological activities of the species.

In comparing results for muscle catalase and respiration in different

[1] Svedberg (1933) finds that the respiratory protein of *Arca pexata* has the same properties (molecular weight, etc.) as that in other invertebrates. This he calls erythrocruorin, as distinct from the hemoglobin of vertebrates.

species, it is assumed that they are representative of whole animals, since muscle makes up the bulk of the tissue in these mollusks. The adductor muscles, moreover, are the organs most directly involved in sustained closure of the shell, in which the greatest economy in oxygen metabolism is required. This point will be discussed later.

Methods

The oxygen consumption of muscles was measured with respirometers of the Thunberg type. Many of the data for certain species, which have been previously published (1930), are incorporated in this paper. Hence the same procedure, of suspending blocks of tissue in air, was followed in later work, in order to obtain results comparable with the others. Single portions of adductor muscle (and of pedal retractor in *Pinna*) were used, thus allowing individual comparisons to be made, except in the case of *Mytilus exustus,* whose small size necessitated the use of muscles from several specimens, bound together as one. The average amount of tissue represented for each species was nearly the same (between 271 and 304 mg.), although the diversity in individual experiments was somewhat greater. The results given were directly calculated as cubic millimeters of oxygen used per gram of tissue per hour (at 27.5° C., not corrected for barometric pressure). Readings were from one to two hours in duration, after allowing one hour for equilibrium to become established. While these may not represent maximal rates of oxygen consumption under conditions favoring perfect diffusion of oxygen into tissues, they are quite adequate for showing the outstanding differences between species. The oxygen taken up by thin sections of muscle, placed in sea water saturated with oxygen and shaken continuously, was determined in certain species, and similar differences in rate of respiration were found as by the above method. Thus, the following figures were obtained: for *Ostrea virginica* (11 experiments), 48.6 cu. mm.; *Venus mercenaria* (6), 33.8 cu. mm.; *V. campechiensis* (4), 28.9 cu. mm.; *Dosinia discus* (16), 62.6 cu. mm.; *Cytherea gigantea* (7), 28.3 cu. mm. Inadequate diffusion of oxygen into the tissue seems to be unimportant as a factor limiting the rate of oxygen consumption, except in *Dosinia,* which has the highest rate of all. Furthermore, the wet weight of the tissue, which forms the basis of these measurements, has been found to be sufficiently reliable for the comparison of results in different species or in types of muscle tissue. All experiments were carried out at 27.5° ($\pm$ 0.3°), which was approximately the mean temperature of the water in which the animals lived during the greater part of the summer.

Catalase determinations were made at the end of every respiration

experiment, on the same tissues. The depletion of catalase in unsectioned muscle, during the four-hour period which elapsed from the time of excision, did not exceed about 10 per cent. It was often much less in species with low muscle catalase content. Hence, the figures in the tables representing catalase and the ratio of oxidation rate to catalase show less extreme differences for species at opposite ends of a series than actually exist, since no correction for depletion was made.

The estimation of catalase was according to the method described in 1930. In all experiments in Bermuda, however, and in those done at Beaufort subsequently, the amounts of extract and of peroxide were kept constant. Thus 20 cc. of a 1 per cent tissue suspension and 2 cc. of 3 per cent hydrogen-peroxide solution were used, giving a 0.27 per cent concentration of H_2O_2 after mixing. The oxygen evolved in 5 minutes was determined at 27°. Owing to the small amount of muscle tissue available in a single specimen, it was found to be impracticable to adjust the concentration of extract so as to decompose a definite percentage of H_2O_2 in a given time. In order to limit decomposition of the reagent to about half, however, a muscle extract of 0.5 per cent concentration was used in all experiments with *Mytilus,* in 13 with *Ostrea,* and in 6 with *Pinna.* Extracts (1.0 per cent) of *Ostrea* and *Pinna* adductors which gave about half decomposition of the peroxide, when diluted to one-half and to one-fourth strength, liberated almost proportionate amounts of oxygen. There was some variation in the activity of these extracts in different concentrations: and on standing, the catalase sometimes increased, then decreased. The average error from these sources is within the limits of $\pm$ 5 per cent. In 10 determinations in *Ostrea* and 2 in *Pinna,* results were obtained by extrapolating from 2-minute readings (using 1 per cent suspensions), and correcting for excess of catalase in relation to H_2O_2. This was unnecessary when the 5-minute readings obtained with the stronger extracts were sufficiently low.

Results

The Carolina species, as listed in Table I, are arranged in general according to their ability to withstand unfavorable conditions, such as exist in poorly aerated aquaria, or under partly anaerobic circumstances in their natural environment. Whether this arrangement conforms to their distribution according to habitat is not always clear, except for the two or three species at each end of the series, because of overlapping. The diverse conditions of environment and of morphology also complicate the situation. *Ostrea* and *Modiolus* are the only non-burrowing species which are periodically exposed at low tides, at which

times the shell remains closed and the animal is subjected to the heat of the sun.[2] *Modiolus* is somewhat less affected by exposure, because it lives on grassy tidal flats, where it may be shaded or partly covered with mud. The other non-burrowing species, *Arca* and *Pecten,* are seldom found above low water level, and show less viability in the laboratory, hence belong lower down in the series. The position of the remaining species, however, must be decided mainly according to their proximity to marshes or to open beaches; their relative size, and activity; the extent to which the animal can withdraw into its shell, and the depth

TABLE I

North Carolina Series

Species	No. of individuals	No. of experiments	Weight of muscle	Oxygen used per g./hr.	Catalase	Ratio $\frac{\text{oxygen}}{\text{catalase}}$ and S.D.
			mg.	cu. mm.		
Ostrea virginica Gmelin	24	29	271	46.4	68.4	0.78 ± 0.28
Modiolus demissus Dillwyn	10	13	291	32.7	31.7	1.00 ± 0.25
Pinna muricata (Linnaeus	12	22	278	40.5	71.3	0.83 ± 0.52
Tagelus gibbus (Spengler)	10	12	280	47.1	33.2	1.43 ± 0.37
Venus mercenaria Linnaeus	30	60	290	38.5	19.8	2.16 ± 0.80
Venus campechiensis Gmelin	10	12	289	35.6	11.5	4.33 ± 1.83
Arca ponderosa Say	10	18	285	29.1	14.1	2.22 ± 0.86
Pecten irradians Lamarck	14	14 striated	281	78.5	19.1	4.53 ± 1.92
		14 plain	275	39.9	31.3	1.32 ± 0.31
Cytherea gigantea Gmelin	10	16	280	28.0	9.5	2.98 ± 0.67
Dosinia discus Reeve	11	15	281	51.4	16.1	3.18 ± 0.65
Cardium magnum Born	8	11	281	30.7	9.5	3.53 ± 1.22
Mactra solidissima, Var. similis Say	6	10	277	41.8	9.5	4.86 ± 1.73

to which it burrows. *Pinna* is a large mussel (up to 30 cm. in length) which inhabits scallop beds, or mud flats on which it is sometimes exposed at low tide. Since the posterior end of the shell protrudes a few centimeters and does not always close tightly, the water within the mantle chamber may be partly lost if the animal is exposed. *Tagelus,* a form having long external siphons, lives in a burrow almost a foot beneath the surface, on sandy mud flats and beaches. Despite the

[2] Although *Ostrea* occurs also in deeper water in certain localities, it is almost restricted to the intertidal zone in Beaufort Harbor. The factors which determine vertical distribution in the oyster are discussed by Prytherch, 1934.

tendency for the shell to gape widely in specimens placed in open aquaria, this species seems to bear mutilation better than *Venus mercenaria,* in which removal of a shell segment without visibly injuring the mantle is usually fatal. In this respect *Tagelus* resembles *Ostrea* and *Pinna.* Six species remain to be considered: they are all closed-shell forms (with retractile siphons), and burrow just beneath the surface of the sand, or, in the case of *Dosinia,* to a depth of about one foot. The species of *Venus* occur on sandy beaches, and on mud bottoms near the marshes as well. Only an occasional specimen of *Cytherea, Dosinia,* or *Cardium* was ever found by the author near the Fisheries Laboratory, and none of *Mactra.* These last four could be collected, however, on a sandy reef (Bird Island) halfway between the laboratory and the entrance to the harbor. The outer part of this shoal, which was out of water only at low tide, was exposed to the action of waves and currents from the open ocean through the harbor inlet. *Cytherea* was usually found well up on the shoal—but not beyond eight or ten inches elevation—in coarse sand or gravel; *Dosinia* just at low water line; *Cardium* also at low water line, but more often under water; *Mactra* only beneath the surface at mean low tide. Shells of *Dosinia* and *Cardium* and of *Mactra solidissima* Chemnitz were plentiful around the inlet and on the ocean beach.

As may be seen from the figures (Table I), much diversity obtains as regards the catalase content of the muscles, whereas the oxygen consumption is more constant. Those species which are intertidal, or which inhabit mud flats, usually have the largest amounts of muscle catalase; those which live almost exclusively along sandy beaches and are not exposed to the air at low tide have least. There are quantitative exceptions to this rule, however, which would suggest that other factors influence distribution in these forms. Thus, in *Dosinia,* the figure for catalase is almost twice as great as that for other species inhabiting a similar environment (*Cardium, Mactra*). In *Modiolus* the amount of catalase is less than one might expect for a species which lives partly exposed between tide levels. May it not be that the magnitude of respiration also affects adjustment to environment?

That the two factors, oxygen consumption and catalase in the tissues, are important in adaptive distribution is indicated when we take into account the ratio of the two. Dividing the first figure by the second, for each individual of a species, the mean numerical quotient (last column of table) gives us a fair indication of the distribution of that species. In the case of *Dosinia,* this quotient serves to place it with the other species found in the same habitat (see above). Likewise, in

Modiolus, the ratio (oxygen: catalase) assigns it to a position higher in the series than the figure for catalase would do.

A comparison of the two species of *Venus* reveals a difference in muscle catalase which is rather striking in view of their similarity and somewhat parallel distribution. Some specimens, in fact, are difficult to identify, and are possibly hybrids. In one individual of *V. campechiensis,* the reading for catalase was found to be 41, although it was less than 14 in each of the others; a fact which also suggests intercrossing, with segregation of physiological characters. The figure for the oxygen/catalase quotient, too, is somewhat higher than one would expect in this species, in view of its wide local distribution. *Venus mercenaria* occurs along sandy beaches, on mud flats, or even in marsh grass areas; it can tolerate semi-stagnant conditions (in terrapin pounds, etc.), and can live on parts of the beach which are not ordinarily covered by water more than one-third of the time. *V. campechiensis,* according to the author's experience, is found in sand, with *Dosinia* and *Cardium;* or

TABLE II

Age Differences in Venus mercenaria: Oxygen Consumption and Catalase

Age group	Number of specimens	Oxygen consumption	Catalase	Ratio $\frac{\text{oxygen}}{\text{catalase}}$ and S.D.
Young clams	16	44.7	17.5	2.68 ± 0.59
Old clams	14	31.4	22.6	1.56 ± 0.53

on mud shoals along the river channels (near the laboratory), at low water line and out at a considerable depth. It seldom occurs more than a few inches above low water level even on the sandy beaches. These differences—in tolerance, and in vertical distribution—help to explain the divergent results obtained in the two species for catalase and O/C quotient.

The relation of *Pecten* to the other species in this series is not clear, because it is highly specialized and lives in no fixed habitat. The main portion of the adductor muscle—comprising more than three-fourths of the whole—consists of cross-striated fibers, which would doubtless play a predominant rôle in the metabolism of the animal. This being true, the scallop should perhaps be placed in a class with the active species (*e.g. Cardium*) characterized by low catalase.

In general, the species in this series which display great variability in shell form etc. are widely distributed and exhibit much diversity in the experimental results (*Ostrea, Pinna, Venus mercenaria, V. campechiensis*); those which show least variability are often restricted to

particular regions or habitats, and give more uniform results in the determinations (*Dosinia, Cytherea, Modiolus*). The relative variation in experimental data is represented fairly well for each species by the standard deviation of the oxygen/catalase quotient in the last column of Table I. In *Pinna*, and to a less extent in *Ostrea*, this variability affects the catalase rather than the oxidation rate, but in the other species it is about equally shared by the two. Special conditions may have influenced the results in certain species, as, for example, the difficulty of finding more than one specimen at a time, and of keeping it in a healthy state until used (*Cardium, Mactra*); the specialized morphology of the adductor in Pecten; or the individual age differences within a species.

The age factor plays a minor rôle in this comparative study of species, because mature specimens were usually required for the experiments. In Venus mercenaria [3] the data for young and old individuals are about equally represented, so that the influence of age on oxygen consumption and catalase can be seen by comparing the averages in the two age groups (Table II); just as in different species, the lower oxygen/catalase quotient is associated with the greater power of survival displayed by mature clams, as compared with young ones, when kept out of water.

The degree of muscular activity exhibited in species of this series is inversely related to the amount of catalase in their muscles; and, on comparing the two diverse types of muscle making up the adductor in *Pecten*, a similar correlation is seen. There is distinctly less catalase in the rapidly contracting, cross-striated muscle of the scallop than in the slow, non-striated part. The rate of oxygen consumption, as determined in the striated muscle after excision, is perhaps higher than the resting value because of a greater responsiveness to stimulation as compared with the smooth muscle. On comparing different species, it may also be said that in the actively burrowing forms there is least muscle catalase (*Cytherea, Cardium, Mactra*); in the more sluggish species which have a foot adapted for digging, there is usually more (*Tagelus, Venus mercenaria, Dosinia*). Although *Dosinia* is found in the same habitat with the first three (active) species, it seems incapable of performing the sudden movements which characterize these, such as leaping in an aquarium.

The four species of Bermuda bivalves (Table III), while morphologically distinct, live in a rather uniform physical environment and

[3] In a previous tabulation of results for *Venus mercenaria* (1930), there were included data for the closely related *V. campechiensis*—Table 2, under August 18 (2) and August 19 (2)—before these species were clearly recognized as distinct. The two specimens were later identified by their shells, and the data are included in this paper under *V. campechiensis*. This change does not materiallly affect the conclusions previously drawn.

hence can be compared directly from the standpoint of their vertical distribution. The average oxygen consumption is nearly identical in each, so that the differences in catalase (and in the oxygen/catalase quotient) are not conditional upon this factor. *Mytilus exustus* is the only intertidal species, occurring at levels up to a foot or more above low tide mark. Its scarcity below this level may be partly due to the depradations of its natural enemies. *Arca occidentalis* and *Chama macrophylla* are frequently found exposed just above low tide, but are much more abundant below this level. *Spondylus americanus* lives in comparatively deep water, and is seldom collected in water less than one foot deep in the inner passages, or in less than half this depth on the outer ocean shore. The author has never seen but one specimen

TABLE III

Bermuda Series

Species, or variety	No. of individuals	No. of experiments	Weight of muscle	Oxygen used per g./hr.	Catalase	Ratio $\frac{\text{oxygen}}{\text{catalase}}$ and S.D.
			mg.	*cu. mm.*		
Mytilus exustus Linnaeus...	44	8	288	23.7	44.3	0.54 ± 0.12*
Arca occidentalis Philippi...	10	20	283	24.4	23.8	1.08 ± 0.32
Chama macrophylla Chemnitz "brown" variety	10	17	285	24.2	24.1	1.24 ± 0.61
Chama macrophylla Chemnitz "red" variety	6	11	277	22.5	10.4	2.18 ± 0.33
Spondylus americanus Lamarck.....	10	20	304	25.8	9.2	3.01 ± 1.00

* Based upon the number of experiments (not individuals, as in the other species).

living, under natural conditions, above low water mark—and then at an exceptionally low tide, on the shaded side of a bridge pile, where the current was swift.

Certain differences in horizontal distribution may be worth noting, as a basis for comparing *Arca* and *Chama* as to their habitats; likewise for the varieties of *Chama macrophylla*. All of the species here represented could be found in Ferry Reach, a rather shallow inner passage, near which the Bermuda Biological Station is located. This communicates with the ocean at a distance of more than a mile, and in the other direction with Castle Harbour. On the outer ocean shore specimens were less numerous, and smaller, doubtless owing to a less abundant food supply. *Mytilus* was collected only in Ferry Reach and its inlets. One of these inlets, serving as an anchorage for the laboratory boats, terminates in a shallow mangrove swamp with a muddy bottom. At low tide many specimens of *Mytilus* were found above water on

rocks a short distance below this mangrove area, and a few of *Arca* at the water line. *Chama* was absent here, but a few specimens were collected farther down the inlet, below the boathouse, where the water is deeper.

Chama macrophylla occurs in Bermuda in abundance, and displays much diversity in form and coloration. The border of the mantle may be deeply pigmented with brown, or various shades of grayish-buff, especially around the siphons. There are evidently different color phases represented in this species, or at least intergrading color combinations; and two of these appear to be distinct. In one the mantle is brown or buff, and the inner shell-surface near the border is of some shade of lavender. In the other variety the siphons are bright red in color, the inner margin of the shell some shade of chestnut.

While investigating the respiration rate and catalase in tissues of this species, it was noticed that, whereas the first was of about equal magnitude in the two color varieties, the readings for catalase were different—although overlapping somewhat (Table III). Since the average result in the red variety was found to be less than half that in the brown variety, it was thought that catalase might be associated with a difference in vertical or regional distribution. No obvious difference in the vertical distribution of the red and brown varieties was observed, but on comparing their relative abundance in distinct localities an interesting result appeared. In a collection of this species made in Ferry Reach, mainly from the Swing Bridge piers, in a total of 155 individuals; 18 (or 11.6 per cent) were of the red variety, 137 (or 88.4 per cent) of the brown variety. A similar count of 43 specimens collected on the outer (north) shore revealed 17 (or 39.5 per cent) of the red, and 26 (or 60.4 per cent) of the brown variety. Whether this proportional difference in distribution represents a partial segregation of the two varieties, based upon their known characteristics, is a matter for conjecture. It is unlikely that color variations would have adaptive significance in relation to such similar habitats. But different degrees of catalase activity of the tissues, perhaps by influencing their underlying oxidative capacity, might determine the relative fitness of individuals for one or the other kind of environment. Animals which live in the open ocean as compared with the inside water passages, are less subjected to daily fluctuations in temperature, and they are exposed to continuous wave action, which, however slight, is a factor of importance. The red variety of *Chama macrophylla,* having less catalase in its muscle tissue than the brown one, is the form which one would expect to find in relatively greater numbers on the outer shore, as was found to be the case in comparing Beaufort species.

Spondylus resembles the scallop (*Pecten*) most closely of all the species studied, except that it is sessile. The two genera are very similar in the physiology and differentiation of their adductor muscles, as well as in gross morphology. In the quick-contracting muscle of *Spondylus gæderopus,* as described by Marceau (1909), the striations are apparently transverse or "en chevrons"; hence they correspond with the cross striations of these fibers in the scallop rather than the oblique striations in the homologous muscle of the oyster and other forms. In *Pecten* the striated muscle was found to have less catalase than the non-striated portion, in every specimen. This is also true for seven individuals out of ten in the case of *Spondylus,* although the differences are small.

Discussion

In the main, the facts in regard to the amount of muscle catalase in different species, in relation to habitat, seem clear; but the interpretation of their significance rests upon our imperfect knowledge of the mechanism of cell oxidations. A detailed comparison of species is not always possible, owing to the diversity of forms studied, the complexity of their environment, and the many sources of experimental error. It may be said, however, that the same order of relationship obtains for the species in the two regions. That is, there is a positive correlation, in each series, between muscle catalase and the ability of species to withstand anoxic conditions. Because of individual exceptions, the ratio between oxygen consumption rate and catalase (O/C quotient, p. 117) seems to represent more accurately this relationship. As suggested previously (1930), catalase possibly plays a protective rôle by regulating physiological oxidation in the muscles of bivalve mollusks, and thus determines the extent to which these are adapted to anærobic life. The presence of much catalase, in the muscles of species which are forced to keep the shell closed for long periods of time, might make for greater economy in the maintenance of tonus, in which only a small increase in metabolism is required. In active species its presence in excess would interfere with sudden demands upon the metabolism of the muscles in rapid, repeated contractions. To the objection that catalase is lacking, or present only in small amounts, in anærobic organisms, it may be said that mollusks are not typical anærobic forms such as derive energy primarily by fermentation.

The function of catalase is considered to be the decomposition of hydrogen-peroxide formed in tissues, thus preventing its accumulation in amounts toxic to the oxidizing enzymes. Since molecular (inactive) oxygen appears in the reaction, it does not promote oxidation directly, but is still available for oxidases to act upon. That catalase has such

a protective function in relation to xanthine-oxidase was demonstrated by Dixon (1925). Moreover, a large amount of catalase, by destroying H_2O_2, retards oxidation with peroxidase, but does not entirely prevent it, since peroxidase reacts with a much lower concentration of peroxide than does catalase (Thurlow, 1925). One can thus explain the rôle of catalase in animals under conditions of oxygen deficiency, by its conserving action on metabolism.

The extension of this investigation to other groups of organisms would doubtless help to clear up many points in regard to their environmental segregation. One such case is that of the occurrence of *Tellina tenuis* between tide marks in the Clyde estuary (Elmhirst, 1932). The greater relative abundance of large specimens at high tide level, and of small ones near low water in an equal area of sand, is understandable on the basis of the greater powers of survival of old individuals out of water, as postulated for *Venus mercenaria* (Table II, and context).

Summary

The catalase and oxygen consumption of the muscles are compared in twelve species of lamellibranchs in North Carolina, and in four in Bermuda. In each region, those species which are intertidal, or which would be subject to partly anærobic conditions, are characterized by larger amounts of muscle catalase than those which live continuously submerged. There is a proportionality between rate of respiration and catalase in muscle for species in a given habitat. The significance of these results is discussed in relation to current views regarding physiological oxidation.

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A METHOD FOR THE DETERMINATION OF AMMONIA IN WATER AND AIR

AUGUST KROGH

(From the Laboratory of Zoöphysiology, Copenhagen University, and the Woods Hole Oceanographic Institution [1]*)*

In the course of a study of the organic material present in solution in sea water, it became necessary to measure also the quantity of nitrogen present as ammonia, and, since the method worked out for this purpose appears to be of wider applicability, especially in oceanography, I propose to describe the method finally arrived at in some detail. It can, with a little practice, be carried out fairly easily and very rapidly even on board ship, a condition which appears to be absolutely essential, since no treatment has been discovered so far which will keep the ammonia of sea water samples at the original level.

— The principle of the method is to make a sample of water containing from 0.05 to 2 γ (gamma = 0.001 mg.) ammonia nitrogen (generally 20 ml.) just alkaline, to distill off in a partial vacuum the ammonia together with about 5 ml. of the water into a small volume of weak acid in which the ammonia can be titrated with naphthyl red according to the method of Teorell (1932).

The Distillation

The following reagents are required:

1. Re-distilled ammonia-free water is prepared from tap water or distilled water containing about 1.5 grams permanganate and 0.1 ml. sulphuric acid per liter by distillation from a Pyrex flask of 2–3 liters capacity. A very effective trap for catching drops is essential and the permanganate is useful mainly because its presence in the trap shows how far drops are carried. About 5 grams of pumice are added to insure quiet boiling. The middle portion of the distillate is taken up in 1-liter glass-stoppered Pyrex bottles and, as a measure of safety, a current of CO_2- and NH_3-free air is blown into the bottle during the distillation. Water for the day's use is poured from the storage bottle into a glass-stoppered bottle of suitable size. This water is used for rinsing and for the preparation of certain further reagents and control samples.

[1] Contribution No. 28.

2. About .25N sodium hydroxide, prepared by dissolving 1 gram of NaOH in 100 ml. NH_3-free water, is used for bringing the water samples to a hydrogen-ion concentration between pH 9 and 11. A sample of the water (20 ml. or a suitable fraction) is titrated in a white porcelain basin with the alkali from a 1- or 2-ml. burette, using cresolphthalein as an indicator. The corresponding amount of alkali or a little more is added to the water sample just before distillation. I have found that even an excess of 50 per cent alkali does not liberate any excess ammonia from the waters so far tested.

3. The ammonia distilled off is taken up in 1 ml. hydrobromic acid about N/100 prepared by adding 0.2 ml. 25 per cent HBr to 133 ml. ammonia-free water. This solution must remain NH_3-free, and if the air in the laboratory shows traces of ammonia it should be protected from contact. Neither the concentration nor the quantity of hydrobromic acid measured off need be determined with any particular accuracy.

The distillation apparatus is constructed upon the general lines laid down by Parnas and Wagner (1921), but certain modifications have been introduced, the most important of which is the use of air at a reduced pressure instead of steam for driving off the ammonia. The distillation flask (Fig. 1, 1) is of Pyrex glass and holds at least 50 ml. in the bulb. It is provided with a standard ground joint of 14–11 mm. Slightly alkaline glycerine is used to lubricate this joint. At least two and preferably three of these flasks should be available for rapid serial determinations.

The connecting piece (2) must also be Pyrex, but the rest of the apparatus can be made from ordinary soda glass. The final 2–3 cm. of the tube (3) should be capillary tubing of 0.5–0.6 mm. diameter constricted at the tip to about 0.1 mm. The part below the condenser can be conveniently constructed from a gas-washing bottle. The bottom is replaced by a rubber stopper through which a glass rod can be pushed up and down to place the collecting bottle (4) at just the right height. For serial determinations a suitable number of these bottles should be available. A metal ring (5) is sealed on to the inside of the bottle with DeKhotinsky cement to prevent the stopper from being sucked in by the vacuum. The stopper and glass rod are kept well lubricated with alkaline glycerine. The 3-way tap (6) connects this part of the apparatus with a vacuum or effective filter pump. Ammonia-free air is admitted to the apparatus through the sulphuric acid bottles (7) and (8). The tube (9) is between 60 and 100 cm. in length and about 0.1 mm. bore and is permanently open. It should provide just enough air for driving over the ammonia when the vacuum is on. The tube (10) is wider. The screw clip (11) is opened only when the distillation is

finished and the pressure raised to that of the atmosphere. When doing a series of distillations, I have always found it necessary to begin with one or more blanks to get rid of traces of NH_3 which will collect even in the closed apparatus. The collecting bottle is charged with 1 ml. N/100 HBr and put in position so that the tip of the tube (3) will just dip into the fluid when the vacuum is put on. About 20 ml. ammonia-free water is put into a distillation flask with a single drop of N/4 NaOH. The evacuation is started as soon as the distillation flask is put on and the burners (11) and (12) ignited. The burner (11) is regulated so as to produce a suitable rate of distillation, usually about 1 ml. per minute. The burner (12) prevents condensation in (2). The bottle (4) is lowered gradually as distillation proceeds so as to minimize splashing, and before it is finished the tube (3) should be clear of the fluid. After distillation of about 5 ml., the gas is turned off, the tap (6) turned so as to shut off the apparatus from the pump without opening it to the atmosphere, and the screw clip (11) opened so as to admit a fairly rapid current of air. It is important to have the gas turned off between determinations as it usually contains appreciable amounts of ammonia. Finally the bottle (4) is taken out and immediately stoppered.

During the distillation of one sample there is time to prepare a fresh collecting bottle and the next sample for distillation. The alkali is added to the latter in the interval during which the pressure in the apparatus is being brought back to the atmospheric level. Following this procedure five determinations can be made in one hour.

The Ammonia Titration

This process has been worked out according to the excellent method introduced by Teorell (1932). Only minor alterations have been made to deal with quantities even smaller than his. The principle of the method is to oxidize the ammonia with a surplus of sodium hypobromite in alkaline solution. After acidification the excess hypobromite is titrated with an acid solution of naphthyl red which becomes decolorized so long as NaOBr is still present.

The necessary reagents are:

1. Sodium hypobromite about n/1,000, made up by dilution from

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Fig. 1. Apparatus for the determination of pre-formed ammonia in water and air.

(1) Distillation flask. (2) Connecting head. (3) Condenser. (4) Receiver. (5) Receiver-holder. (6) Three-way tap connecting with vacuum. (7) and (8) Sulphuric acid wash bottles. (9) Fine capillary for constant admission of air. (10) Clamp for returning to atmospheric pressure. (12) -*a*-, -*b*-, micro-burners. (13) Bottle with syringe-pipette for hypobromite solution. (14) Hydrobromite acid. (15) Absorber for the determination of ammonia in air.

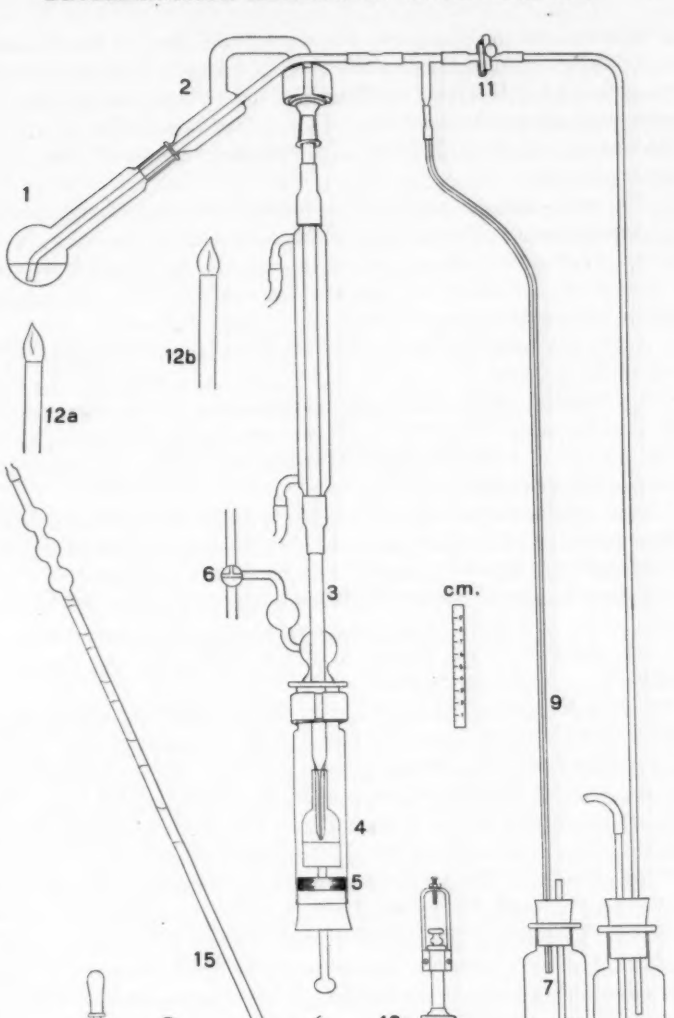

FIG. 1

n/10 stock solution (2.5 grams NaOH, 1.25 ml. fluid bromine in water to 500 ml.). The reaction between NaOBr and NH_3 must take place at a pH of 8.5–9 and the n/1,000 sodium hypobromite is therefore made sufficiently alkaline with 2n Na_2CO_3 to give this pH when about 1 ml. is added to 1 ml. of n/100 HBr. I have found 5 ml. of 2N Na_2CO_3 per 500 ml. suitable.

The stock solution will remain constant indefinitely when kept at a low temperature. The dilution, which must be kept in a dark brown bottle, is not quite constant, but can be used for weeks. I have found it convenient to transfer the quantity for each day's use into a smaller brown bottle and to keep the stock in complete darkness.

2. Hydrobromic acid about 30 cc. 25 per cent diluted with ammonia-free water to 200 cc.

3. Naphthyl red n/2,000 made up by mixing in the order indicated 30 cc. glacial acetic acid, 3.75 cc. 89 per cent H_3PO_4, 1.9 cc. 25 per cent HBr, 10.5 cc. of a freshly prepared 0.1 per cent solution of naphthyl red in water, water to 500 cc.

This solution keeps well, but a small amount of a brown precipitate which interferes with the titration is often formed, and the best plan is to filter off each day the quantity necessary for the day's use.

4. Ammonium sulphate. The stock solution contains 0.4716 gram $(NH_4)_2SO_4$ in 1 liter. This is kept protected against evaporation.

One milliliter of this diluted to 100 ml. with NH_3-free water will contain 1 γ nitrogen per milliliter.

It is convenient to measure off the hypobromite for each titration with a syringe pipette mounted in the bottle as shown in Fig. 1 (13). The volume of the pipette need not be just 1 ml., but is regulated so as to correspond to something between 1.8 and 2.0 ml. naphthyl red. The hydrobromic acid I keep in a small bottle (14) with a ground-in rubber-capped pipette which will deliver approximately 0.2 ml.

The titration is made from a 2-ml. burette divided in 200 parts. It is necessary to do each day 2 to 4 blanks and 2 to 4 titrations of a known amount of ammonia. The results depend to some extent upon the temperature, and when the room temperature undergoes considerable variation more constant results are obtained if the solutions to be titrated are brought to an approximately constant temperature in a water basin.

To do a blank, measure off in the titrating bottle 1 ml. n/100 HBr, add about 5 ml. NH_3-free water, bring to the desired temperature, add hypobromite and shake gently; fill up the burette with naphthyl red to the zero point. This takes about the half minute required for the reaction between hypobromite and ammonia to become complete. In order to secure uniform results constant timing should be adhered to. To the sample is now added 0.2 ml. 4 per cent hydrobromic acid; it is again

shaken up and titrated from the burette to a just visible rose color. It is advisable to use a comparison bottle with water and just enough naphthyl red to produce this color. The titration must not be made too rapidly, but should take at least 1 minute. For the determination of the NH_3 equivalent of the naphthyl red, 1 or 2 ml. of the ammonium sulphate solution is added to the n/100 HBr and enough NH_3-free water to bring the volume to 5–6 ml. The mixture is treated as above and titrated. To test the absorption of NH_3 during distillation, ammonium sulphate is added to 20 ml. water and distilled off.

Example:

	ml. naphthyl red solution
Blanks titrated directly	1.96, 1.95
Blanks distilled	1.95, 1.95
Ammonium sulphate (2 γ N) directly	0.89
Ammonium sulphate (2 γ N) distilled	0.89

2 γ N = 1.06 ml. naphthyl red solution

For the determination of ammonia in fluids where less than 1 γ is regularly to be expected, it may be worthwhile to reduce the volume of hypobromite to .5 ml. The accuracy to be obtained in the single determinations of a series is of the order 0.04 γ N which corresponds by the distillation of 20 ml. to ± 2 mg. ammonia nitrogen per cubic meter.

The Determination of Ammonia in Air

An apparatus like the one shown (Fig. 1, 15) is suitable. The glass tube is 6 mm. in internal diameter and about 50 cm. long up to the lower of the three bulbs. The top is connected to a water bottle of suitable capacity (4 to 10 liters) from which the water is allowed to run out at a rate of 4 liters per hour or less. The bottle is charged with 1 ml. N/100 HBr and 4–6 ml. NH_3-free water and the whole is placed in a slanting position so that the absorbing fluid moves fairly regularly right up to the bulbs, thus providing a constantly renewed absorbing surface of about 100 $cm.^2$ Titration is performed as above in the absorbing bottle. The air in a laboratory for ammonia determination should contain less than 0.1 γ N per liter.

Summary

A method and apparatus for the determination of ammonia in water are described. The ammonia is carried over by a simple vacuum distillation into hydrobromic acid in which it is determined by the naphthyl red titration of added hypobromite.

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METHODS FOR THE DETERMINATION OF DISSOLVED ORGANIC CARBON AND NITROGEN IN SEA WATER

AUGUST KROGH AND ANCEL KEYS

(From the Laboratory of Zoöphysiology, Copenhagen University, and the Woods Hole Oceanographic Institution[1]*)*

Introduction

Certain problems in the nutrition of marine animals and in the general economy of organic life in the sea involve the determination of organic matter present in solution in the water. The methods worked out by the senior writer for the determination of dissolved organic matter in fresh waters are of no avail. In these methods (Krogh, 1930) the organic material is concentrated by evaporation of a large sample of the water and the organic material determined by combustion of the dry residue, but the sea water salts, present in quantities several thousand times as large as the organic material, make any attempt in this direction futile. All we can hope to accomplish by means of the resources at present available are determinations—more or less approximate—of the nitrogen and carbon present in organic combination, and these simplified analytical problems are what we have endeavored to solve.

Assuming according to the scanty and not especially convincing estimates to be found in the literature (Atkins, 1922; Brandt, 1916–20; Gran and Rund, 1926; Harvey, 1925; Natterer, 1892–1900; Vernon, 1898) that we should have to deal with quantities of at most a few milligrams per liter of water, it has been our endeavor to work out microchemical methods of handling water samples of small volume. Large samples are in many cases not available, and, more important, the necessary time and expense in preparing large samples for analysis would be a serious drawback.

The Determination of Organic Nitrogen

A large number of trials were made with micro Kjeldahl methods, but they all failed. The quantity of sulphuric acid necessary for the Kjeldahl treatment becomes relatively very large on account of the salts, and it was not found possible to obtain or prepare sulphuric acid of sufficient purity. All samples examined would, after the Kjeldahl

[1] Contribution No. 47.

heating in ammonia-free air, give off a variable amount of ammonia when distilled after addition of excess sodium hydroxide. The sulphuric acid was treated in various ways to get rid of this "organic" nitrogen and in the final procedure evaporated in a current of oxygen. The mixture of vapor and oxygen was taken through a quartz tube containing platinum heated along a length of about 10 cm. to 800–900° C. and along a further length to about 400° C. From the distillate supposed to contain some NH_3 a portion was again distilled off in a high

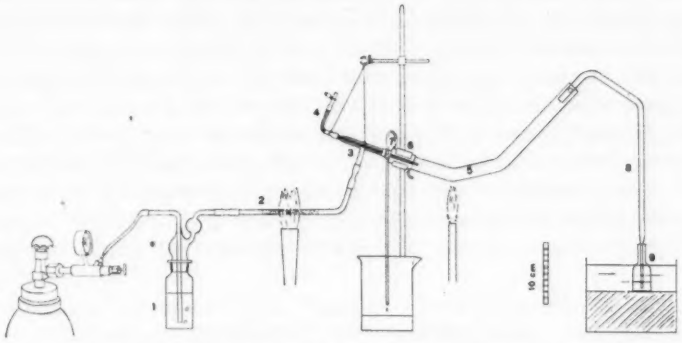

FIG. 1. Apparatus for the determination of dissolved organic nitrogen in sea water.

(1) Sulphuric acid wash bottle.
(2) Quartz reduction tube.
(3) Glass connecting head.
(4) Tube for introduction of sea water sample.
(5) Silver tube.
(6) Water jacket.
(7) Cooling water outlet.
(8) Silver condensing tube.
(9) Receiver.

vacuum and tested, but even with this acid reliable results could not be obtained, because the acid itself would in heating yield variable amounts of nitrogen of the order of 0.5 to 2 γ (0.0005 to 0.002 mg.) N per milliliter.

The old Will-Warrentrapp method was suggested to us by Professor Biilmann and we finally succeeded in working out a modification of it suitable to our needs.

In this method the organic material is heated to about 500° C. with alkali in an atmosphere of hydrogen and, in the absence of oxygen, most of the organic nitrogen is liberated as NH_3, while a small part may be driven off as amines. No type of glass and not even quartz will stand this treatment more than a few times and we therefore do the combus-

tion in a silver tube. The ammonia (+ amine) is taken up in N/100 HBr, combined with sodium hyprobromite, and the excess hyprobromite titrated according to the method of Teorell described in detail by Krogh in the preceding paper in this journal.

The apparatus is shown in Fig. 1. Hydrogen from a cylinder is bubbled through sulphuric acid (1) to remove any traces of NH_3 and to indicate the rate of flow which should be of the order of 10 to 20 ml. per minute.

The purification of the hydrogen before it enters the silver combustion tube may be accomplished in several ways. The simplest arrangement is to bubble it through sulphuric acid to remove any traces of NH_3 and then to pass it through a quartz tube (2) with metallic copper as a contact substance heated to a dull red heat to remove traces of oxygen. The frequent presence of arsenic and antimony traces in the copper, however, require caution in its use. A safer procedure is to bubble the gas through sulphuric acid, then through a long absorption train containing pyrogallic acid, again through sulphuric acid, and finally to pass it through the quartz tube over metallic platinum foil heated to a red heat.[2]

The connecting piece (3) is glass. The solution for combustion is added from a pipette through (4). A small part of the silver combustion tube (5) is water-jacketed and the clamp (6) grips the water jacket (7), which is a short piece of brass tubing. The current of water is regulated so that the water in the jacket will just boil when the maximum heat is applied to the combustion tube.

An analysis is performed as follows. A pellet of sodium hydroxide is placed in the combustion tube (5) which is then connected up to (3) and the system is washed out with hydrogen. The water sample—usually 5 ml.—is run in through (4). A collecting bottle with 1 ml. of N/100 HBr is placed in a basin with cold water so that the narrow tip of the silver tube will just dip into the acid. The combustion tube is heated gently at first to boil off the water and finally ignited to a very dull red heat which can be observed only when the room is dark. When sea water is analysed some ammonia will become occluded in the salts, and it is therefore necessary, after cooling, to run in an additional 2 ml. of "blank" water which should contain no nitrogen, or at all events only a known small amount, and evaporate this also. Finally the whole tube including (8) is heated (below the incineration point) by moving the flame so as to bring every trace of water into the collecting bottle (9). We have found that two determinations can be made in series without

[2] We are indebted to Dr. Homer P. Smith for having worked out this second procedure.

opening the combustion tube, but thereupon the salts must be washed out. To do this (3) is disconnected, a little nitrogen-free water poured in and heated to near boiling. The water jacket is emptied. The tube is turned slightly up and down in the clamp and the water poured out. This is repeated once. A fresh pellet of sodium hydroxide is put in and after connecting up, 2 ml. of N-free water is added. When this has been distilled off and the tube ignited it is ready for the next two determinations.

The most serious difficulty in this technique is the preparation of sufficiently pure water for the washing operations. In Copenhagen water from the municipal supply could be distilled off first from an alkaline solution of permanganate and again from sulphuric acid and permanganate. In Woods Hole and in Cambridge, Massachusetts, acceptable water was prepared by distillation from strongly alkaline permanganate and then from very dilute phosphoric acid. Both distillations were carried out in a current of washed air in all-Pyrex stills.

The distillate is tested both for free ammonia and for "organic" nitrogen by the combustion method. One sample of water gave the following results:

Test for free ammonia—Titration of 1 ml. NaBrO with

2.5 ml. water directly,	1.89_5	Mean = 1.89 ml. Napthyl red
5.0 ml. water directly,	1.89_5	
7.5 ml. water directly,	1.89	
2.5 ml. water + 2.25 γ N,	0.66	

Hence 1 ml. napthyl red $= \frac{2.25}{1.89-0.66} = 1.83\,\gamma$ N.

Test for "organic" N—Titration of 1 ml. NaBrO with

2.5 ml. water "burnt," 1.85, equivalent to 0.04 ml. napthyl red,
5.0 ml. water "burnt," 1.80, equivalent to 0.09 ml. napthyl red,
7.5 ml. water "burnt," 1.77, equivalent to 0.12 ml. napthyl red,

15 ml. water "burnt," 0.25 ml. napthyl red.

Hence 1 ml. of the "blank" water contains 0.030 γ N (0.030 mg. per liter). The single determinations show that within limits of error of the titration the nitrogen obtained as ammonia is proportional to the quantity of redistilled water used.

When 2 ml. of "blank" water are used in a combustion of sea water they necessitate a correction of 0.06 nitrogen or 0.03 ml. napthyl red to be subtracted from the value obtained. Distillation of deep sea water gave a satisfactory result, *i.e.*, a low and constant "blank" value.

A series of determinations on the same sea water taken in varied quantities gave the following results:

Blank value	1.90		1.88	ml. napthyl red	
2.25 γ N	0.65$_5$		0.67		
	1.24$_5$		1.21	1 ml. napthyl red = 1.83 γ N	
					Sum
Vol. of sea water, ml.	5.25	5.1	3.0	7.0	20.35
ml. napthyl red used (corrected for NH_3 in "blank" water)	0.73	0.72	0.47	1.005	2.925
γN in 10 cc.	2.54	2.58	2.86	2.62	2.64, or 0.264 mg./l.

From this quantity the amount present as preformed NH_3 must be subtracted to arrive at a figure for "organic" nitrogen.

Some control determinations on dilute solutions of glycine, creatine, and urea averaged about 5 per cent too high, but the correction for the organic matter in the distilled water used was somewhat uncertain. Analyses on acetamide and cocaine hydrochloride solutions also proved the efficiency of the method. Nitrates do not affect the results in the least. Table I, below, summarizes the results from a number of control experiments. In addition some results on various distilled waters are given.

TABLE I

Control analyses for organic nitrogen. Concentrations are in γ of nitrogen per liter (mg. per cubic meter).

Cocaine solutions		Nitrate solutions		Ordinary laboratory dist. H_2O	Conductivity water
Known	Found	Known	Found		
800	792	250	0	90	44
"	820	"	10	94	58
"	836	"	−6	85	40
"	798	"	14	87	46
"	778	"	4		
"	816	"	12		
400	410				
"	400				
"	388				

THE DETERMINATION OF ORGANIC CARBON

We have worked out a wet combustion method not very different in principle from that described by Lieb and Krainick (1931).

The water sample is acidified to drive out preformed CO_2. Most of the chlorine is precipitated out with thallium sulphate and the water thereupon evaporated to dryness. The dry residue is oxidized with

chromic and sulphuric acids in a current of carefully washed air which carries the evolved CO_2 and CO through an oxidizing combustion tube and then into a baryta solution which ultimately is titrated with HCl.

The necessary reagents are prepared as follows:

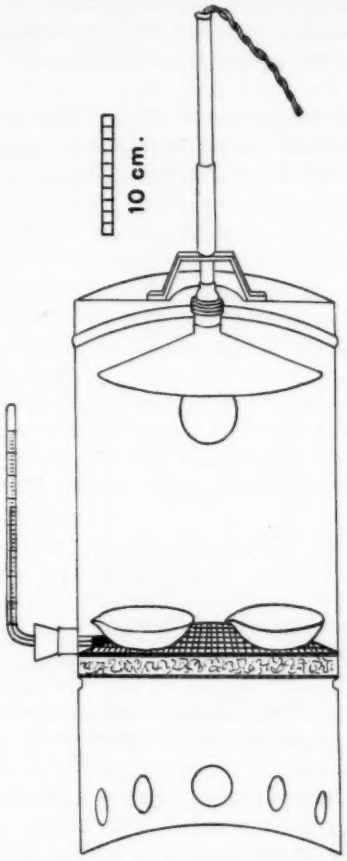

FIG. 2. Radiation evaporation chamber.

(1) Phosphoric acid dissolved in pure redistilled water and diluted to about n/10.

(2) Methyl red used as indicator.

(3) Thallium sulphate (Tl_2SO_4). The purest preparation available is melted in a crucible and stirred well so as to remove by combustion

any trace of organic material. We use for stirring a glass tube connected with an oxygen cylinder, but this may not be necessary. After cooling, the salt is carefully powdered and preserved for use in a wide-necked glass-stoppered bottle.[3]

(4) Chromate-sulphuric acid for oxidation: 10 grams potassium bichromate, 4 grams silver chromate, and 1 gram chromic acid are mixed in a crucible and melted together. The resulting solid block is pulverized.

To 50 cc. redistilled water and 50 cc. of the purest sulphuric acid we add 6 grams of this chromate mixture and 3 grams cerium sulphate. The mixture is put into the storage bottle (Fig. 3), placed in a glycerine

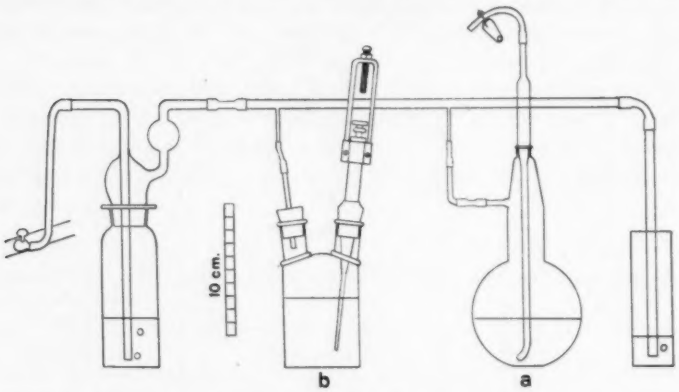

FIG. 3. Arrangement for storing reagents.
(a) Ceric-chromic sulphuric acid.
(b) Baryta solution and syringe-pipette.

bath at 125°–130°, and heated under aeration with CO_2-free air for about 24 hours. The mixture is kept protected against CO_2, as shown in Fig. 3 (*a*).

(5) Potassium bichromate c.p. kept as a fine powder in a glass-stoppered bottle.

[3] The thallium chloride precipitate from the analyses can be recovered as thallous sulphate easily. The precipitate is gathered into a large round-bottomed centrifuge tube in which it is washed and centrifuged several times, minimal amounts of cold distilled water being used for each washing. The washed precipitate is placed in a casserole with a 50 per cent excess of pure sulphuric acid and the whole cooked at 400° C. for 24 hours in a hood. The casserole should be covered with a cover propped in place so as to give a free clearance of about an eighth of an inch all around. After this digestion the temperature is raised to boil off all the sulphuric acid (450° C.) for about 6 hours. After cooling in a desiccator, the solid mass of thallous sulphate is broken out and carefully pulverized in a mortar. It is now ready for use.

(6) Hydrochloric acid N/3 used for titration in a Rehberg microburette. One cubic millimeter of this acid corresponds to 2 γ carbon. The stock of acid should be kept in a bottle provided with a ground cap over the stopper to insure against evaporation, which will often take place to a quite appreciable extent from ordinary glass-stoppered bottles.

(7) Baryta solution about N/12 to N/15 prepared with 0.5 per cent $BaCl_2$ (and cresolphthalein[4]). The baryta solution is kept in a bottle protected as shown in Fig. 3 (*b*) and with a 0.5 ml. syringe-pipette mounted on top. The volume of baryta delivered by the pipette is regulated so as to correspond to between 90 and 100 mm.^{3} of the n/3 acid.

The apparatus and procedure is as follows: The salinity of the water is determined. (A hydrometer will give sufficient accuracy and all samples of ocean water can be taken as having a salinity of 35‰.) From 10 to 25 cc. of the sea water are measured off in a white porcelain basin, methyl red is added, and standard phosphoric acid is run in from a 2 cc. burette or measuring pipette until a persistent red color is obtained. The corresponding volume of H_3PO_4 N/10 is added to the samples to be analyzed. These samples (we use 25 ml. each) are measured off in centrifuge bottles as shown (Fig. 4, *c*) and thallium sulphate is added. The quantity of Tl_2SO_4 necessary depends upon the salinity. We allow an excess of about 30 per cent, which means that for a 25 ml. sample of ordinary sea water 2.5 grams of pure thallous sulphate is sufficient; a greater excess is not harmful.

The vessels are shaken in a suitable shaking machine for 10 or more hours. The shaking need not be violent. Good results have been obtained by putting in two glass balls and placing the bottles on a slowly rotating vertical wheel. After the shaking the bottles are centrifuged for a few minutes and 20 ml. of the supernatant fluid can now be pipetted off for the analysis.

These samples are evaporated down to dryness in porcelain basins. Several methods have been tried. With evaporation in an oven at 105°, splashing is difficult to control and there is some danger of losing organic matter. Evaporation in a vacuum oven is a slow process and some splashing generally occurs. The simple radiating oven shown in Fig. 2 has been found very useful. It is made from tinplate, the surface of which reflects much light and heat. A rapid current of air is maintained by the heat of the 60-watt lamp, and as the air is filtered through the layer of cotton wool, contamination with dust is effectively guarded against. The temperature may rise to 70° C., but not until the samples are almost dry, which takes about 4 hours. Eight hours will ensure

[4] Enough cresolphthalein is added to give a deep red color in the bottle and to give a pale but very distinct red when placed in a tube of 6-mm. bore.

thorough desiccation. If it is necessary to leave the samples for some time after drying, they should be kept well protected against moisture and dust—preferably in a desiccator.

The white crust of salts should be fairly easy to scrape off. This is transferred with great care to a combustion flask with side arm. We put in a couple of glass balls and one spoonful (about 0.2 gram) of potassium bichromate. The combustion flasks are cleaned with thiosulphate solution, then with bichromate cleaning fluid, and finally are

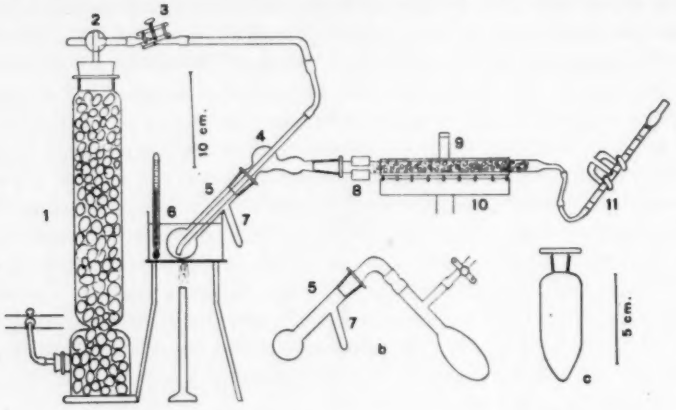

FIG. 4. Combustion apparatus for the determination of dissolved organic carbon in sea water.

(1) Soda lime tower.
(2) Three-way tap.
(3) Pinchcock air-flow regulator.
(4) Connecting head.
(5) Combustion flask.
(6) Glycerine bath.
(7) Side arm to hold oxidizing reagent.
(8) Pyrex combustion tube.
(9) Tube for thermometer in metal jacket.
(10) Long burner.
(11) Baryta tube.
(b) Arrangement for drying flasks rapidly.
(c) Stoppered centrifuge tube.

rinsed just before use with a little redistilled water which is poured out as completely as possible and dried. This is done quickly by immersion in hot water after connection with a filter pump (Fig. 4, *b*).

When the combustion material and bichromate have been thoroughly mixed, the suitable volume of chromate-acid is put into the side arm (7, Fig. 4). For a sample of ocean water 3 ml. are necessary. The standard ground joint is lubricated by just touching it with the tip of the pipette delivering the chromate acid.

The flask is connected with the combustion apparatus shown in Fig. 4, and the beaker with glycerine (6) used as a bath is put in position. Air at a low pressure (*e.g.* from a reversed filter pump acting as a blower) passes through moist soda lime in (1) to remove CO_2 and sweeps through the entire system.

The combustion tube (9 in Fig. 4) is arranged to remove completely all last traces of halogens or halogen acids and to ensure that any CO will be converted to CO_2. In traversing it the air passes, successively, asbestos shreds, lead chromate dispersed in asbestos, cupric oxide, asbestos, platinum foil, asbestos impregnated with finely divided metallic silver, and finally asbestos shreds. The combustion tube is made from Pyrex or other hard glass. A length of 14 cm. is surrounded by a copper tube (9) heated by the burner (10). A thermometer can be placed in (9) and the burner regulated once for all so as to give a temperature between 400 and 450° C., which is maintained throughout when analyses are in progress.

At first a fairly rapid current is maintained to wash out the system for about 10 minutes. The flow of air is regulated by the clip (3) and may be observed in the absorption tube (11), which at this stage contains a single drop of water. During this period the glycerine bath is slowly warming.

When the temperature in the glycerine bath has reached about 80–90° C., the air current is reduced so far that baryta solution can be put into the absorber (11) without danger of its being spilled out. The combustion flask is now turned so as to transfer a little of the chromate-acid in the side arm to the bulb. This procedure must be carried out with some caution because it lowers the temperature and absorbs any water vapor present and is therefore liable to suck back baryta from (11) into (8), which of course will spoil the analysis. When all the chromate-acid has been transferred it is often necessary to mix the contents in the bulb by several times revolving the tube (5). When a temperature of 120° is reached the combustion is continued for 30 minutes, which will be sufficient to drive off all the CO_2 formed.

A clip is now placed on the rubber tube of the absorber, which is transferred to a Rehberg micro-burette stand and connected with a slow current of CO_2-free air. During the titration the tip of the micro-burette dips about halfway down in the tube and the baryta is kept moving up by means of the air current. When the end point is almost reached the absorber is removed from the burette, the solution is rinsed about in the narrow portion of the absorber and afterwards in the bulb, the absorber is replaced, and the titration is finished. The end point can be judged with an accuracy of 0.1 mm.[3]

Blank determinations on the chromate-acid alone and with thallium sulphate are necessary. The chromate-acid will always give off a certain amount of CO_2 corresponding to 2–10 γ carbon, and since this liberation depends to some extent on time and temperature, it is essential that the blanks should be treated in every way like the analysis proper. The average of several blank titrations should be used for the determinations.

Example: baryta titrated directly 93.0, 93.0 mm.^{3} N/3 HCl. Blanks with 3 ml. CrA 88.1, 87.8, 89.5, 87.4, 89.3, 89.3 = 88.6 average. Samples of sea water, 25 meters, samples 1–2 untreated, 2–4 sterilized with several drops of $NaHSO_3$. The untreated samples require 0.50 ml. of H_3PO_4 N/10, the others are already acid. The same amount of Tl_2SO_4 added to each.

Titrations, mm.^{3} N/3 HCl:

Untreated 67.0, 63.1; sterilized 64.4, 63.6.

Average 64.5; blank 88.6.

Carbon found $2 \times 24.1\ \gamma$ in 20 ml. = 2.41 mg./liter.

The accuracy to be obtained does not exceed 0.1 mg./liter. When, as in Copenhagen, there is much coal dust in the air, it is essential that the combustion apparatus be kept closed except during the short intervals of time when combustion flasks are changed.

A recent series of blanks with the new mixture, containing cerium sulphate, but prepared from ordinary pure sulphuric acid, gave the following values for 0.5 ml. chromate-acid: 0.7, 1.2, 1.0, 0.7, corresponding to 3.6 γ C per ml. These low blank values indicate that the preparation of special sulphuric acid may not be necessary.

Organic Matter in Colloidal and in True Solution

The problem of the amount, if any, of organic material in sea water present in the colloidal state is of considerable importance. It is to be expected that the disintegration of organisms will yield a considerable proportion of colloidal material, but whether this will remain in the colloidal state for any length of time is at present unknown. The nitrogen determinations described above will comprise everything present, whether suspended, colloidal, or dissolved, but the precipitation of chlorides, which is a necessary initial step in the carbon determinations, will throw down all suspended particles and may be expected to remove also a variable proportion of colloids.

Colloids can be removed from water samples by ultra-filtration, but care must be exercised to guard against contamination from the filters and the filtering apparatus. We have used ultrafilters from "Mem-

branfilter Gesellschaft," Göttingen, Germany, designated as 70 minutes, which means that at a pressure difference of 1 atm. 100 ml. of pure water will be filtered through 100 sq. cm. in 70 minutes.

Our filtering apparatus is a modification of the Thiessen Ultrafiltration apparatus and it will stand a pressure of about 5 atm. The filtering surface of 4 cm.² will therefore allow the filtration of about 15 ml./hour. We generally pass the water first through a 25–35 sec. filter which will remove all suspended matter including bacteria.

If the water contains more than a trace of colloids the rate of filtration through a 70-minute filter at a constant pressure will gradually become reduced, but in the few experiments so far made with ocean water no decrease in rate was observed and no difference could be found between carbon or nitrogen values before and after ultra-filtration.

The detailed results of the application of these methods to sea water collected from various localities and depths will be presented in separate papers.

Summary

A method for the determination of organic nitrogen in solution in sea water is described in detail. The principle of Will-Warrentrap, involving digestion at 500° C. with caustic soda in an atmosphere of hydrogen, is used. The sensitivity of the method is roughly 0.0003 mg. N, giving an accuracy in sea water of about 5 per cent in 5- or 10-ml. samples.

A wet combustion method for the analytical determination of dissolved organic carbon in sea water is presented. Halides are removed with thallous sulphate, the evaporated sea water residue is oxidized by means of ceric-chromic sulphuric acid, and the evolved carbon dioxide eventually determined by acid titration of baryta solution through which the gases have passed. The accuracy approaches 0.1 mg. carbon per liter.

Differentiation between organic matter in colloidal and in true solution is made by analyses before and after ultra-filtration, using " Membranfilter Gesellschaft " collodion filters and a modification of the Thiessen ultrafiltration apparatus.

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STUDIES IN THE LIFE HISTORIES OF EUGLENIDA[1]

IV. A Comparison of the Structure and Division of Distigma Proteus Ehrenberg and Astasia Dangeardi Lemm. A Study in Phylogeny[2]

JAMES B. LACKEY[3]

Introduction

The Euglenida are perhaps the most widely investigated of the non-pathogenic flagellates. This interest is due to their beauty, wide distribution, size, amenity to culture, and their varied modes of life, ranging from the holophytic through the saprophytic and parasitic to the holozoic. Despite our long acquaintance with them, they occupy an anomalous position in taxonomic studies, both among zoölogists and botanists, and the classification within the group is unstable, as shown by Rhodes (1926), Hall and Jahn (1929), Calkins (1933), the writer (1934), and others. In 1931, while investigating the relations between environmental factors and mitotic behavior in cells under a Grant-in-Aid from the National Research Council, the writer found an easy method of cultivating these and other Euglenida; so the present paper is an indirect contribution to that study and is a further attempt to add to our knowledge of the Euglenida.

Material and Methods

The organisms studied were obtained in activated sludge from the Tenafly, N. J., Sewage Disposal Plant, where they occur in small numbers. They were cultivated on various tap water dilutions of autoclaved wheat in Petri dishes, under a film of paraffin oil. Cover glasses with zoögleal films containing them were fixed in Schaudinn's, Gilson's, and Flemming's fluids and stained with iron hæmatoxylin, eosin being occasionally used as a counterstain.

Description of the Animals

Distigma proteus (Ehrenberg) is briefly described by Ehrenberg (1838), Walton (1915), and Pascher (1913), so no lengthy descrip-

[1] Journal Series paper N. J. Agricultural Experiment Station, New Brunswick, N. J., Dept. Water and Sewage Research.

[2] This is the first of a series of papers prepared through the assistance of a Grant-in-Aid from the National Research Council.

[3] Research Zoölogist, Dept. Water and Sewage Research.

tion need be given here; but in addition to the characteristics noted by these writers there are certain features which might be called to attention as additional diagnostic features. Figure 1 shows the normal type of

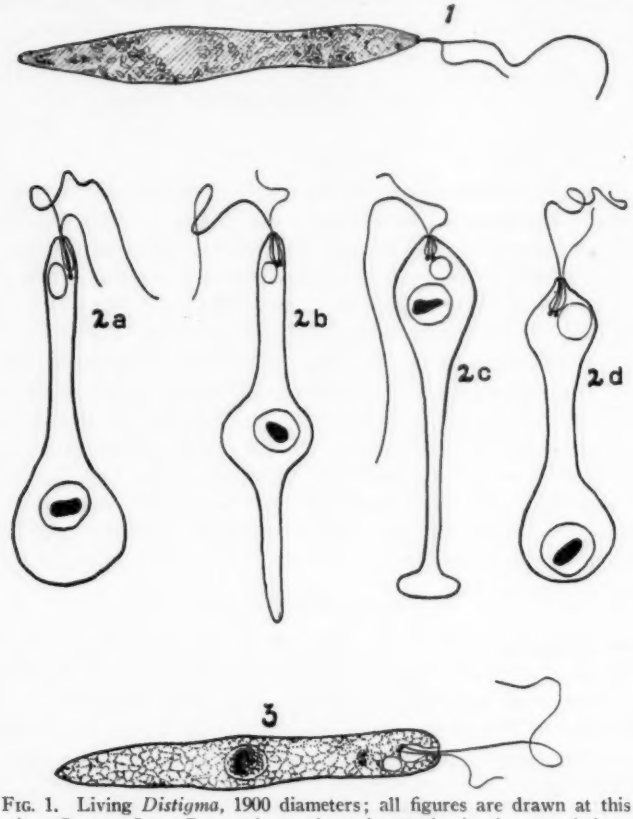

FIG. 1. Living *Distigma*, 1900 diameters; all figures are drawn at this magnification; Spencer Lens Co. apochromatic equipment having been used throughout. Note: faint gullet, reservoir, and adjacent contractile vacuole. The small size of the paramylum bodies is characteristic.

FIG. 2. Sucessive shapes assumed by a living animal as an enlargement of the posterior end of the body moves forward.

FIG. 3. Optical section of an animal after fixation in Gilson, stained with iron hæmatoxylin. Note: flagella, blepharoplasts, the endosome and accessory smaller granule in the nucleus, gullet, reservoir and contractile vacuole. Below the contractile vacuole are two cytoplasmic granules whose function is unknown.

living animal, and Fig. 2 shows the characteristic contortions which are more pronounced in this organism than in any other euglenoid with which this writer is familiar.

The paramylum granules are notably constant in size and shape. Except for the one mentioned below, they seem to be the only cytoplasmic inclusions, for wherever they are absent the cytoplasm is finely granular and homogeneous.

The gullet, reservoir, and contractile vacuole are shown in Fig. 3. The reservoir is quite small, and the gullet is short. One, or often two, small contractile vacuoles are formed beneath it. The two flagella enter the gullet and pass into the side wall of either the gullet or the reservoir. They do not bifurcate and each ends in a blepharoplast adjacent to the wall. Studies thus far have failed to reveal any fibrils from the blepharoplasts to any other parts of the cell. After fixations with Gilson's fluid, one or two cytoplasmic granules (Fig. 3) are found in 85 per cent or more of individuals studied, but may not be found after Schaudinn's, Flemming's, or Benda's fluids. In some they are between the nucleus and the anterior end; in others, posterior to the nucleus. It is possible that these granules are comparable to the one described by Baker (1926) as a parabasal homologue, but it has not been possible to trace them in division. Gilson's fluid also gives a different idea of nuclear structure, in that it shows an additional intranuclear body, smaller than the endosome, but present in about 98 per cent of the fixed animals. The percentages given are based on counts of one hundred individuals encountered one after the other in moving the slide across the field of vision, and are illustrative of the thousands on each cover glass. Multiple endosomes are common in the Euglenidæ, as reported by the writer (1929), Ratcliffe (1927), and others, but the selective fixation evidenced here is unique, and indicates the presence within the nucleus of two "endosomal" bodies which are of different composition. The endosome, as revealed by Schaudinn's and Flemming's fluid, is single and generally spherical or ovoid, devoid of granules or internal vesicles.

The nuclear membrane is thin, or lacking, but the nuclear vesicle is clearly defined, because the spaces in the chromatin reticulum are devoid of visible contents, apparently being filled with nuclear sap. Tschenzoff (1916) believed the nucleus in *Euglena viridis* to be a vesicle whose boundary did not constitute a definite wall. The chromatin is in the form of granules, apparently forming a permanent spireme, the granules composing linear aggregates.

The length of the animal varies, but such great variations as mentioned by Walton and Pascher were encountered only in animals which were elongating or contracting excessively. The average length was 80 microns.

No evidence was found indicating other than a saprozoic mode of existence.

Encystment was noted in some of the cultures, but was never very common. The animals first rounded up and became quiescent while retaining their flagella. The vacuoles functioned for some time, but finally a thick wall was formed. At this time the cyst is round (Fig. 4),

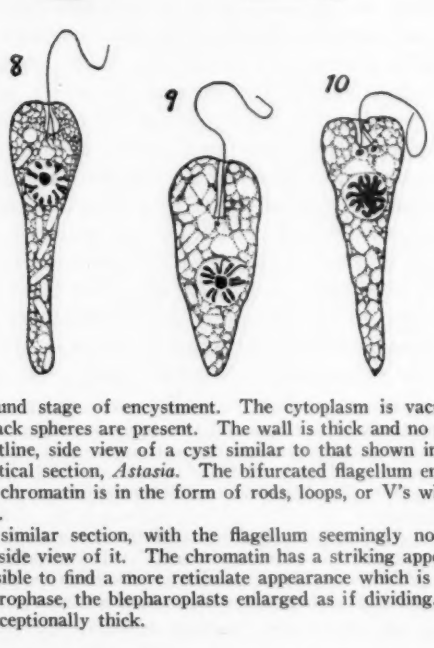

FIG. 4. Round stage of encystment. The cytoplasm is vacuolated, and numerous small black spheres are present. The wall is thick and no pores are visible.

FIG. 6. Outline, side view of a cyst similar to that shown in Fig. 5.

FIG. 8. Optical section, *Astasia*. The bifurcated flagellum ends in two blepharoplasts. The chromatin is in the form of rods, loops, or V's which do not seem to be connected.

FIG. 9. A similar section, with the flagellum seemingly not bifurcated. In reality this is a side view of it. The chromatin has a striking appearance of paired rods. It is possible to find a more reticulate appearance which is not figured.

FIG. 10. Prophase, the blepharoplasts enlarged as if dividing. The chromatin elements are exceptionally thick.

and the staining power is retained, but shortly after, the cyst becomes a yellow rectangular or pentagonal shape (Figs. 5 and 6) with thick walls and pores at the corners. It contains many paramylum granules, and the contents stain faintly or not at all. In cysts in which nuclear structure can be made out, there is no endosome, and the chromatin seems to be aggregated principally against the nuclear membrane. No attempt has been made to follow the history of encysted animals. As

far as the writer is aware, cysts of this type have not heretofore been reported for any of the Euglenidæ.

The *Astasia* dealt with herein was obtained from the same source and cultured in the same manner and often with the *Distigma*. It appears to conform most closely to the description of *A. dangeardi*, as given by Walton and Lemmermann, and is placed in that species. The animals are extremely metabolic, whether free-swimming or creeping, and may be club-shaped one moment, a flattened oval the next. The gullet-reservoir vacuole system is so reduced as to be all but invisible in the living or stained animals at a magnification of 1900 diameters. The number of paramylum granules is small, from 8 to 12 (Fig. 7), and there is more clear cytoplasm in the animals observed than in the *Distigma*. There is a single flagellum almost as long as the body, which

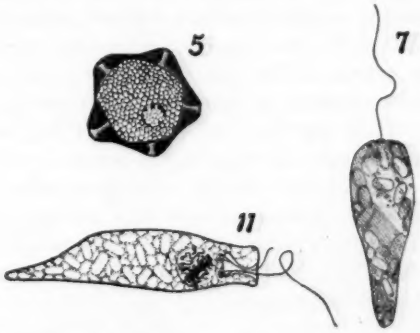

FIG. 5. Pentagonal cyst, from above. The thick yellow wall has 5 pores. The black spheres have disappeared, the cytoplasm has become homogeneous and the nucleus, devoid of an endosome, has its chromatin peripherally located.

FIG. 7. Living *Astasia*. The shape is typical and gullet, reservoir, contractile vacuole, and nuclear vesicle are faintly shown as well as paramylum granules, cytoplasmic granules, and the striations of the pellicle.

FIG. 11. Prophase, the new flagella well developed. The elongate endosome is surrounded by a thin spireme, so entangled that it is barely possible to see the endosome.

bifurcates in the reservoir and ends in two blepharoplasts. Hall and Jahn (1929) report that the flagellum does not bifurcate, but on slides containing thousands of individuals the writer has had no difficulty in finding plentiful evidence of bifurcation in the vegetative as well as the division stages.

The posteriorly located nucleus seems universally to have a single endosome and the chromatin is in the form of threads (Fig. 8), loops or V's, a condition usually indicative of a permanent spireme stage.

No cytoplasmic inclusions other than the one type of paramylum

bodies have been demonstrated in this study. The animals are apparently saprozoic and are facultative anaerobes. No cysts have been identified by the writer and, contrary to the statement of Walton, division is by longitudinal binary fission in the active state.

Division of Living Astasia

From cytological examination, it had been ascertained that the anterior end of a dividing *Astasia* was relatively square in shape. Watching a living culture at a magnification of 950 diameters, an individual trapped in a small space was observed at 7:20 P.M. to have this square end. This indicated approximately the metaphase. One flagellum was visible in the right half, which also showed a very small gullet and reservoir. All the paramylum was crowded into the posterior end of the body, indicating that the nucleus now occupied the anterior end. Within five minutes, a shallow median furrow appeared and deepened rapidly. At 8:00 P.M. the daughter individuals were three-fourths separated. The animal moved quite a bit within its confines, but no violent contortions occurred, as happens in *Peranema*. The new flagellum of the left half was first visible at 7:50 P.M., but thereafter, it rapidly increased in length, although it was not as apparent as the original one when the two cells parted. The paramylum granules were pushed back until the constriction was half through the body, then, nuclear division evidently being complete, the granules streamed into each daughter half. At 8:10 P.M. the two cells separated suddenly and active swimming about began.

Division in Fixed and Stained Animals

The above account is substantiated in stained preparations. The beginning of mitosis is indicated by a thickening of the chromatin threads, and the formation of the endosome into a rod. The anterior end becomes square and the nucleus migrates into it. The chromatin strands become so thick as to almost fill the nuclear space. In this organism the thickness and close spacing of the chromatin threads surrounding the endosomes is so marked as to all but obscure it and no idea of the shape of the spireme or chromosomes can be had even in very much destained specimens. They do not form an orderly pattern about the endosome (Fig. 11), as they are too long and their ends are too much entangled. Because of these conditions no definite metaphase arrangement can be made out. In the succeeding stage (Figs. 12, 13) the endosome is surrounded by a cordon of long, straight, but irregularly beaded rods, at least some of them undergoing an apparent transverse median division. Such a chromosome configuration is shown in almost all of the

figures of workers who have described the division of Euglenida. Dangeard (1901) reported this, terming the rods "chromospires"; Bělař (1916) has reported a similar case of Astasia; and Khawkine (1886),

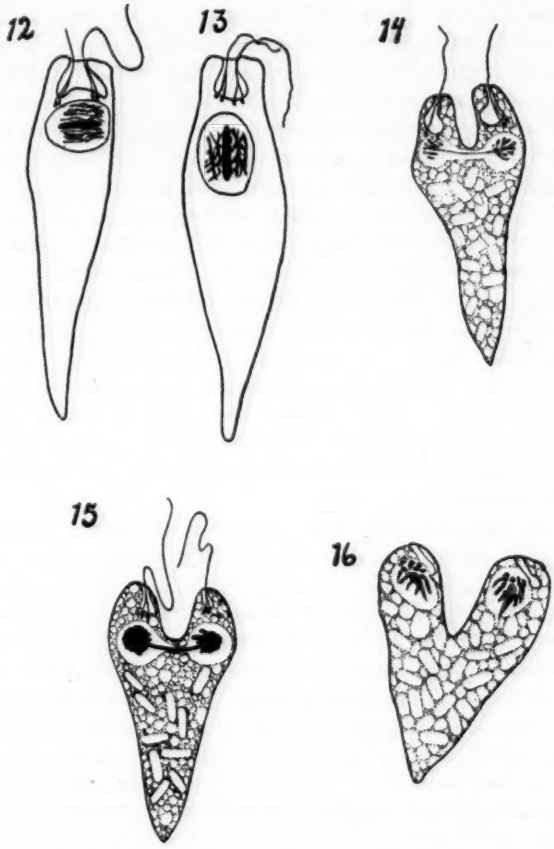

Fig. 12. A metaphase in which the chromosomes have become straight rods, which will shortly break transversely, the breaks being at the apex point of the original V-figure.

Fig. 13. Same, but the long axis of the nucleus parallel to that of the animal. Such figures are often found but no intermediate angles between this and that of Fig. 12 have been found.

Fig. 14. Anaphase. There are 12 visible daughter chromosomes at one pole, 11 at the other.

Fig. 15. Anaphase, in which the chromosomes are clumped.

Fig. 16. Telophase. Each daughter nucleus contains 12 chromosomes. No flagellla were visible in this organism.

Tschenzoff (1916), Baker (1926), and Ratcliffe (1927) have shown similar conditions for species of *Euglena;* Hall and Powell (1928) and Brown (1930) for *Peranema;* and the writer (1929) for *Entosiphon.*

The daughter groups of chromosomes move to the opposite ends of the nucleus quickly. From the observations on the living animal, it is judged that from the splitting apart of the chromosomes until the nuclear reorganization takes only about an hour. These daughter chromosomes do not become vacuolated; they simply form a tangled mass about the end of the endosome until individuality is lost (Fig. 15). It is only after the V's have become practically straight, *i.e.* when the two daughter chromosomes are still attached to each other at the end, that a correct count of their number can be made, and even then a dividing nucleus must be found in which the long axis of the endosome is vertical. Better counts may be made from anaphase or telophase nuclei. From such nuclei (Figs. 14, 16) it is believed that the number of chromosomes is 12.

The endosome, here as in other Euglenida, acts as the central axis of the division figure. In the prophases it becomes a straight rod (Figs. 11 to 16), then elongates and begins to pull apart in the middle, coming to resemble a double-headed club, which is finally pinched in two as the nuclear membrane separates. Its two pointed rods persist for some time, but eventually they shorten and enter into the reorganization of the nucleus.

The behavior of the flagellum and kinetic elements is typical. One set is retained by one daughter cell, the other getting a new set. The old blepharoplasts each bud off a new one, and the two pairs slowly separate. Evidently the roots of the new flagellum growing out from the daughter blepharoplasts unite after they have grown out a short distance. Direct evidence on this is very hard to obtain, but it is indicated in Figs. 10 and 12. The blepharoplasts in *Astasia* can hardly be considered as the division centers because of their position, which is not at the ends of the division figure, but more on the anterior face of the persistent nuclear membrane. Additional force is lent this interpretation by the retention of one flagellum and its activity, while the animal is dividing.

Nuclear reorganization is also typical. The daughter chromosomes aggregate about the end of the shortening endosome, and insensibly change into the condition characteristic of interkinesis. Throughout the whole process the cell has retained its elongate shape and is active.

Division in Distigma

In this animal the cell rounds up and undergoes the euglenoid movements characteristic of *Peranema* in division. The nucleus often pre-

pares for division before the kinetic organelles. The endosome elongates; simultaneously the chromatin forms a thin spireme (Fig. 17) of ever increasing thickness. It is not a smooth spireme, but a beaded one,

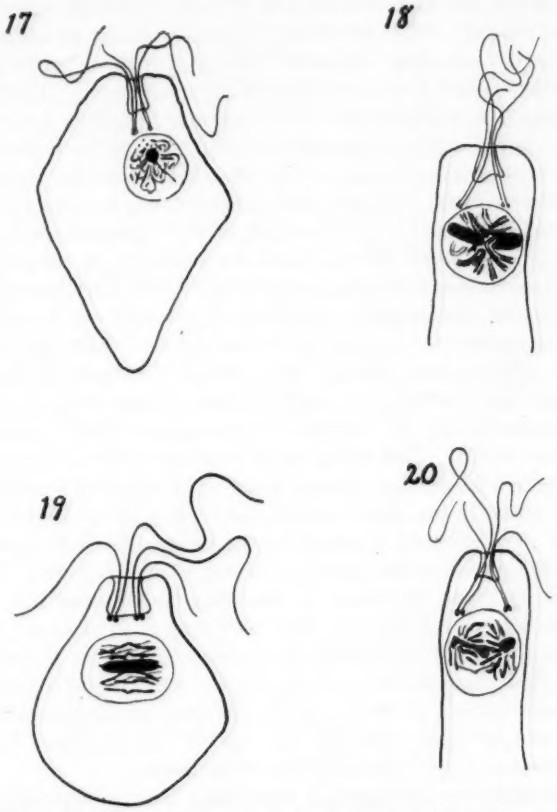

FIG. 17. Optical section transverse to the long axis of the endosome of *Distigma*. The thin spireme is well shown. Four flagella are already present.

FIG. 18. The metaphase plate in Distigma. The chromosomes are longitudinally split, and are U's or V's.

FIG. 19. Metaphase plate in Distigma. The chromosomes have straightened out to encircle the endosome as a cordon of beaded rods.

FIG. 20. Anaphase in *Distigma*. At least one of the chromosomes has not yet parted at the apex of the V. The blepharoplasts are manifestly not the poles of the division figure.

the chromosomes being of varying sizes. However, the beading is much less pronounced than when the chromosomes are fully formed. The threads are either more widely spaced than those of *Astasia* at this stage,

or their length and number of twists and turns is less, for whereas in a stained *Astasia* the nucleus is a mass of stained black threads, in *Distigma* the individual threads can be discerned. These threads now shorten and at the same time become thicker. Then the chromosomes are differentiated. They are oriented about the center of the endosome (Fig. 18) in a somewhat tangential plane at this time, but their length prevents the formation of a well-defined metaphase plate. From studies of this stage, it is indicated that the chromosomes are long irregular rods and that they undergo a longitudinal split. Such a split may be observed in several chromosomes in Fig. 18. When this split occurs could not be determined in *Distigma;* the spireme is too tenuous and the resulting nucleus (Fig. 17) gives no indication of paired strands of chromomeres. Tschenzoff (1916) found an anaphasic or telophasic split for the chromosomes of *Euglena viridis,* which infers an interphasic persistence of the chromosomes. Nothing of the sort can be seen here; the first appearance of a split is at the metaphase. Evidently the chromosomes split longitudinally and a separation of daughter halves begins at one end, thus forming Y's and V's which gradually open out altogether, separation of the daughter chromosomes finally occurring at the apex of the V. This would mean a terminal traction fiber attachment in cells with spindles, such as some sea urchin egg cleavages. In *Distigma* many of the chromosomes pass to the poles as straight rods just as if they had such terminal attachments and they thus present a remarkable parallel, in this anastral division, to an astral one. They do not change in length, but about the time they have formed their cordon about the endosome (Fig. 19), that body begins to elongate, and the chromosomes part in the middle, simulating a transverse division. No pairing of the chromosomes is to be found, but it would be difficult to see, because, instead of being radially disposed to the endosome, they are now parallel to it. At this time, and in the anaphase (Fig. 22) 16 chromosomes can be counted in optical sections.

As the endosome elongates, it acts like a heavy but pliable rod; if cell constriction has begun, it may bend more and more before the advancing depression until it becomes U-shaped. The chromosomes remain grouped about its two ends, undergoing few changes other than a gradual shortening. They remain separate and deeply staining after the endosome has pinched in two. Reorganization of the daughter nuclei, however, lags far behind cell division; and often daughter cells may be seen (Fig. 23) in which the pointed endosome, still appearing to pierce the nuclear membrane posteriorly, and the daughter chromosomes may still be found, marking that cell as a young individual. No especial significance seems to be attached to this, however, and the nucleus eventually assumes a typical interkinetic appearance.

The behavior of the kinetic elements is somewhat different from that in *Astasia*. The two flagella each end in a single blepharoplast, and in division each blepharoplast divides or buds off a new one. The new

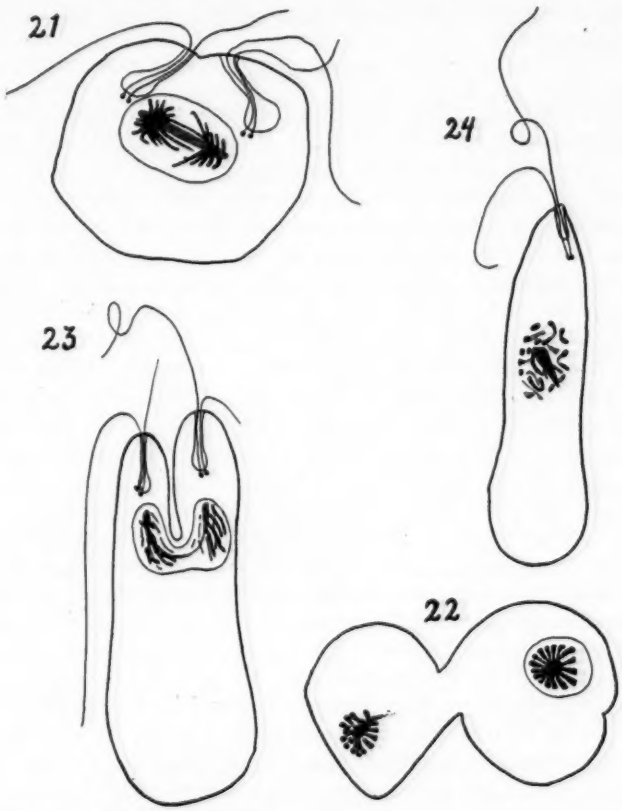

FIG. 21. Same stage, long type.

FIG. 22. Optical section through two daughter nuclei of *Distigma* at anaphase. Sixteen chromosomes can be counted.

FIG. 23. Telophase, *Distigma*. The endosome is U-shaped, and the chromosomes are still distinguishable.

FIG. 24. A "young" *Distigma*. Chromosomes still distinguishable, and the endosome still drawn to a point.

flagella then grow out quickly from the new blepharoplasts. The two pairs have assumed positions approximately at opposite poles of the division figure at the time of separation of the chromosomes. As in *Astasia* they never act as division centers, and logically it is difficult to

conceive how one persistent pair of blepharoplasts and another pair budded off during the process of division could act as division centers, despite the fact that blepharoplasts generally are stated to have such a function. No such assumption is necessary, however, for the progress of nuclear division and development of the kinetic apparatus are synchronous.

Discussion

The method of nuclear division in the Euglenida has been of absorbing interest since the early works of Khawkine (1886), and Keuten (1895), and the later work of Dangeard (1901) in this field. Since the Protista might reasonably be expected to offer examples of primitive modes of division, as pointed out by Wilson (1930), it is not surprising that chromospire formation in the Euglenida as described by Dangeard offered a division seemingly primitive. Recent research, however, has tended to prove that the beaded chromosomes of the Euglenida in reality divide by a longitudinal split and not by a transverse break in a straight rod-like chromosome. Tschenzoff (1916) offered evidence of this, and Baker (1926). Hall (1923), Hall and Powell (1928), Ratcliffe (1927), the writer (1929), and others have shown that the chromosomes are generally of such length that they usually make up a tangled mass in the prophases and do not form an orderly, equatorial metaphase plate. However, as they split longitudinally, they begin to straighten out, so that the endosome comes to be surrounded by a cordon of elongated rods. If most of the chromosomes were rods which began to split at one end, they would eventually give a configuration such as that pictured by Dangeard in his account of chromospire formation and transverse splitting in various Euglenida, or by Bělař in *Astasia levis*. If the chromosomes were V's or if the split began in the middle, such shapes would be formed as figured by Tschenzoff, Baker, et al. In the present paper the writer believes he has shown with certainty that elongate chromosomes are formed just as in many other organisms; that a sort of metaphase plate is formed about the endosome, but the alignment of its chromosomes in an equatorial plane is obscured or prevented by their tenuousness, entanglement, and lack of spindle fibers; that each chromosome undergoes a transverse split which in most of them begins at one end and results in the formation of V-shaped structures as the split progresses; that, as the split nears the end of the chromosome, the chromosome becomes a straight rod; and that the final separation of the two daughter halves is the apparent transverse split so often seen. Bělař has shown this apparent transverse split very well in his figures of the division of *Astasia levis*, and they may be seen in the figures of other workers. If the writer's interpretation be true, how-

ever, there is nothing particularly primitive about the partition of the chromatin to the daughter cells in these Euglenida.

The position of the nucleus in dividing astasias is remarkably constant. In the interkinetic organism it may be in the anterior, median, or posterior part of the animal, but in division it is always in the anterior end in close approximation to the kinetic apparatus. If it were not for the fact that nuclear division progresses as rapidly as the division and outgrowth of kinetic elements, it would seem as if the blepharoplasts must indeed serve as division centers. A similar position is taken by the nucleus of *Euglena agilis,* according to Baker, and may be correlated in this organism with the origin of the kinetic complex from the endosome of the nucleus. However, the writer does not find any connection with, or origin from, the nucleus of the kinetic apparatus in *Astasia,* so the constancy of position is thus far without significance.

One of the most striking features in mitosis in the Euglenida is the behavior of the endosome. Spindles are present in none of the Euglenida thus far described, and it is generally stated that the endosome is the division center. In a literal sense this is true and there is little variation in the form and behavior of the endosome among the various Euglenidæ whose division has been studied. The chief variation seems to be that the endosome may change early or late in mitosis; in *Astasia,* elongation begins as soon as there is any evidence of mitotic activity; in *Distigma,* elongation occurs only when the spireme is well organized and the daughter flagella are already in evidence; and in *Euglena viridis,* according to Tschenzoff, only after the chromosomes are well formed. In forms with multiple endosomes, the elongation is occasionally shared by more than one of the fragments. Hall and Powell (1928) picture such a case in *Peranema,* and the writer has seen such instances in this same organism. The peculiarity in *Peranema* is that, when the endosome consists of more than one part, the additional fragment elongates toward one pole only, and does not divide.

In *Astasia,* the endosome quickly shortens and rounds up in the final stages of division into the shape typical of interkinesis, while in *Distigma,* the endosome retains its long-drawn-out pointed shape for some time after separation of the daughter cells and may even pierce the nuclear vesicle to enter the cytoplasm. The whole process of nuclear reorganization is very slow in *Distigma,* and one may frequently distinguish "young" cells by their chromosomes and pointed endosomes. If there is any significance in this, it is not apparent, but it does serve to call attention to the support afforded the chromosomes by the endosomes, for they retain their individuality for a long time. The writer is of the opinion that the endosome in the Euglenida takes the place of

the spindle in division, and that it acts in no wise as a centriole, and does not contain a centriole in any euglenid studied by the writer.

There seems to be no definite structure which can be called a division " center " in the Euglenida. There are some accounts of centrioles, but it has not been shown that they initiate mitotic phenomena. In the Euglenida investigated by the writer, the endosome might serve as the division center in *Entosiphon,* where the blepharoplasts sometimes do not divide until metaphase, and likewise in *Astasia;* the blepharoplasts might serve this function in *Peranema;* while there is no distinguishable center in *Distigma.* Nor is the endosome a division center in *Euglena viridis,* according to Tschenzoff. In anastral mitosis, it is perhaps gratuitous to seek a division center; for there is hardly a cell structure more dynamic than chromatin itself. But it does offer difficulties to a " blepharoplast-centriole " concept, not to have blepharoplasts serving as such centers.

In view of the admittedly unsatisfactory classification of the Euglenida, the findings in this paper are offered as an aid in this field. Hall and Jahn (1929) have recently pointed out that probably the best basis for a satisfactory classification will be cytological, and they show that the Euglenidæ investigated by them all have a bifurcated flagellum, whereas the Astasiidæ do not have such a bifurcation. The writer believes, however, from a critical cytological study, that the flagellum in *Astasia dangeardi does* bifurcate. If this be true, their criterion for placing colorless forms with one flagellum in the Astasiidæ is no longer valid. However, the green members—*Euglena, Phacus, Trachelomonas,* and *Lepocinclis,* which the writer has investigated—also show the characteristic thickening on one of the flagellar roots (Fig. 25), familiar to students of the group. This structure is readily shown following fixation in Flemming's fluid and staining with iron hæmatoxylin and it is present in euglenas which have been kept either in the dark or in a rich organic medium so that they have lost their chlorophyll. If this condition is true of all the green members, and of these which have merely lost their chlorophyll, it is still possible to divide the order into families on a cytological basis. All those green members and the uniflagellate forms, whose flagellum bifurcates, and in which there is a lens-like thickening on one root of it, should be placed in the family Euglenidæ. All uniflagellate forms with or without a bifurcated root to the flagellum, but no lens-like thickening, should be placed in the family Astasiidæ, while all biflagellate and polyflagellate forms, none of which apparently show any bifurcation of the flagellum, should be included in the family Heteronemideæ. The only present change would be the necessity of transferring *Peranema* to the Heteronemideæ, close

to *Heteronema,* which it greatly resembles. This scheme of classification would not invalidate the suggestions of Hall and Jahn except to make advisable the retention of the stigma-bearing astasias in the genus *Astasia* pending further cytological examination.

At present, the only colorless form with a bifurcated flagellum, aside from *Euglena,* seems to be *Astasia dangeardi,* and the writer differs from Hall and Jahn in regard to this point. There is, however, certainly no lens-like thickening on the roots or root of its flagellum, so its position is secured.

FIG. 25. Euglena sp. A specimen fixed in Flemming and stained with iron hæmatoxylin to show the nuclear structure and the bifurcated flagellum. Each root ends in a blepharoplast adjacent to the floor of the reservoir. There is a heavy discoid thickening on the flagellum just before it bifurcates.

Moreover, such a classification represents a phyl netic series. The ancestral form (Fig. 26*a*) was probably a euglenid with chlorophyll, and a flagellum with a single root; its possession of the lens-like body is problematical, but it seems possible that the stigma is a more recently developed structure than chloroplasts. Some euglenas, notably *E. deses,* have many granules of the same material as the stigma, which move about in the cytoplasm by cyclosis or Brownian movement or both. If the stigma is a recently developed structure, so, probably, is the flagellar swelling.

From this ancestral form, the present Euglenidæ (Fig. 26*b*) arose when the flagellum split at its base, possibly as a result of abnormal behavior in division, the blepharoplast dividing, and either the flagellum attempting to divide, or the outgrowth from the new flagellum uniting with the old one. In another place the writer will show that such behavior actually occurs in *Entosiphon*.

The third step in the series resulted when *Euglena* lost its chlorophyll, and therefore the structures necessary for orientation toward a source of light. The resulting organism (Fig. 26*c*) is of the *Astasia* type. Loss of chlorophyll is so common for some species of *Euglena* that comment is unnecessary.

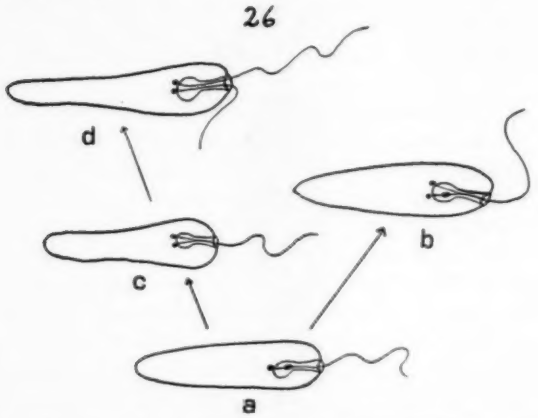

FIG. 26. The suggested phytogenetic history of the Euglenida. The primitive organism, green and with an undivided flagellum is seen at *a*. The present green forms generally conform to *b*, while the uniflagellate colorless forms such as *Astasia dangeardi* are represented by *c* and the bi- and polyflagellate colorless forms, the Heteronemideæ, represented by *d*, are derived from a form like *c*.

Finally, if an organism of the *Astasia dangeardi* type, that is, with a bifurcated flagellum root and no flagellar thickening, should have the flagellum completely bifurcated, either by a split along its entire length or by failure of the two roots to unite, the biflagellate colorless forms (Fig. 26*d*) would be produced. It is not difficult to conceive that such an evolutionary process of derivation has taken place, or even to derive such forms as *Eutreptia* and *Euglenamorpha*, and indeed Wenrich (1924) asserts that evolutionary processes are evident in his studies of the latter form, although he considers that duplication of the flagella is occurring. Such a series, however, emphasizes the interrelationships of the members of the order, and the rational basis of classifying them.

Summary

1. The structures of living and stained *Distigma proteus* Ehbg. and *Astasia dangeardi* Lemm. are shown and compared.

2. Formation of yellow pentagonal cysts is noted for *Distigma*.

3. *Distigma* has 16 chromosomes, *Astasia* has 12. In both animals they are elongate and somewhat beaded. They form irregular metaphase plates and by a separation of halves of the longitudinal chromosomes, surround the endosome with a cordon of rod-like chromosomes.

4. The final parting of the two daughter chromosomes is as a rule at one end, but it simulates a transverse break.

5. Individual chromosomes may persist in the nuclei of daughter cells of *D. proteus* even after separation, but in *A. dangeardi* reorganization of the nuclei is complete by the time the cells separate.

6. Mitosis is anastral in these organisms, there being no centrioles, asters, or spindles.

7. The endosome seems to take the place of the spindle.

8. The blepharoplasts do not function as division centers, but do divide, or bud off daughter blepharoplasts.

9. One daughter cell gets an old kinetic complex (flagella, blepharoplasts) and the other part gets new kinetic organelles, the one or two flagella growing out from the blepharoplasts.

10. It is suggested that the *Euglena* with a bifurcated flagellum on which is a discoid thickening, and the Astasiidæ having a bifurcated flagellum with no discoid thickening came from an ancestral form whose flagellum did not have two roots, while the complete splitting of the bifurcated flagellum of the Astasiidæ gave rise to the Heteronemideæ, with two or more non-bifurcated flagella.

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THE EFFECT OF VERY DILUTE ETHYL ALCOHOL UPON THE RATE OF CELL DIVISION

Results of Measurements on Eggs of the Marine Worm, Urechis

D. M. WHITAKER

(From the Department of Zoölogy and the Hopkins Marine Station, Stanford University)

Most investigations of the effect of very dilute alcohol upon the rate of cell division have been carried out with *Paramecium,* but *Spirostomum, Stentor,* and *Stylonychia* have also been used. The narcotizing effect of higher concentrations of alcohol which suppress cell division has been studied on marine eggs by a number of investigators,[1] but I do not know of any attempts before now to see if there is in these forms a stimulating effect of more dilute subnarcotizing concentrations. The concentrations of alcohol which inhibit cell division in the Protozoa and in marine eggs are of the same order of magnitude, and are similar to the concentrations which cause narcosis of a large variety of animal cells and tissues: *i.e.,* about 2–5 per cent, depending on the species and the exposure time (see 3, 4, 7, 8, 9, 11, 12).

Experiments upon Protozoa

Calkins and Lieb (2) found that the division rate of *Paramecium* in $\frac{1}{25}$ per cent to $\frac{1}{16}$ per cent ethyl alcohol was increased 30 per cent or more compared with controls. The effect lasted at least a month and the general vitality of the culture was improved. Estabrook (4), however, found that concentrations of 1 per cent or less had no effect whatsoever upon the growth or division rate.[2] Woodruff (14), using pure line cultures, confirmed Calkins and Lieb in finding that $\frac{1}{25}$ per cent alcohol increased the division rate of *Paramecium,* but the stimulation was temporary (*e.g.,* 5 to 30 days) unless reëstablished by doubling the alcoholic strength to $\frac{1}{12}$ per cent, and again to $\frac{1}{6}$ per cent. Similar results were obtained with *Stylonychia.* Summarizing a large amount of data, Woodruff concludes that minute doses of alcohol decrease the rate of cell division at one stage of the life cycle and increase it (temporarily) at another.

Matheny (11) found that *Paramecia* were retarded in 3 per cent

[1] For references, see (7, 8 and 9).

[2] Retardation and inhibition occurred in 2 per cent and more.

alcohol but that in concentrations ranging from 1/100 per cent to 2 per cent there was no effect. Bills (1) found that dilute methyl, ethyl, propyl and butyl alcohol all improved cultures of *Paramecium* (pure lines of *P. caudatum* and *P. aurelia*) which were deficient in food. Ethyl alcohol was best. When food was deficient in the medium, in which two kinds of bacteria predominated, 1 per cent ethyl alcohol both postponed starvation of the culture and death, and revived starving cultures.

Daniel (3) found that a certain type of *Stentor* and that *Spirostomum* thrive in 1 per cent alcohol, with the division rate stimulated. Another type of *Stentor* showed ciliary stimulation but no increase in division rate. Concentrations of 2 per cent and 3 per cent caused death in a few hours.

These results of studies on the Protozoa are in large measure inconsistent and contradictory. But this is perhaps to be expected in view of the complexity of these organisms and especially because of the variability of the media employed. It was not possible to use sterile, constant, reproducible media when these experiments were performed. Even so, there are some points of agreement throughout: (1) Alcoholic concentrations of 2 per cent or somewhat more retard cell division and growth. (2) Concentrations of 1 per cent to 1/25 per cent either are without effect or else stimulate cell division and growth.[3]

Purpose of Experiments Here Reported

For testing the effect of sub-narcotizing concentrations of alcohol upon the rate of cell division, a suitable marine egg has several advantages compared with the Protozoa:

(1) The medium (sea water) is constant.

(2) There is no nutrition problem. Food is self-contained and in excess.[4] There is little reason, therefore, to suppose that alcohol will act here as a stimulant to division by compensating for food deficiency.

(3) As J. Gray (6) has pointed out, protozoan cell division is more immediately related to growth than is necessarily the early division of the egg cell, which may continue as a mere subdivision of materials, not necessarily dependent upon increase of protoplasmic mass.

[3] Growth as well as division when the size of the daughter cells is not reduced in proportion to the increased division rate.

[4] That the rate of cell division is limited by factors other than the concentration of available food is suggested for the sea urchin egg by the results of Loeb and Wasteneys (10). That the same relations hold in *Urechis* is an assumption which is reasonable at present. The fact that eggs develop to the gastrula stage before taking in food from the outside, and (Gray (6)) that successive early cleavages proceed without diminishing cleavage rate is further evidence.

Method

The eggs of the marine worm, *Urechis caupo* (Fisher and MacGinitie (5)) have been used. One large female contains many millions of eggs which may be removed from time to time for weeks or months. All eggs used were taken from the same female, and were fertilized with sperm from the same male. The adults were kept in separate aquaria of running sea water and both yielded apparently perfect gametes two months after experiments were terminated.

All conditions were standardized as much as possible. A large automatic water bath thermostat was used which contained heating and cooling units and which varied in temperature $\pm$ 0.01° C. throughout the whole period of the experiments. Due to an error in recording, it is not absolutely certain whether the temperature of the bath was 12.93° C. $\pm$ 0.01 or 13.93° C. $\pm$ 0.01. It was almost certainly the latter, and in any case was the same throughout all measurements.

Moist chambers were made from Petri dishes in which a paraffin partition, as high as the wall of the dish, divided the bottom of each Petri vessel into two compartments. In each experiment three 1-cc. glass dishes with flat bottoms were placed in each compartment, the three on one side being the experimental vessels, the other three being the controls. Sea water covered the bottom of the compartment containing the controls, and an alcoholic solution of the concentration being used experimentally covered the bottom of the experimental compartment of the moist chamber. The cover of the Petri dish was sealed on with vaseline which also sealed the top of the paraffin partition. The moist chambers were then totally submerged and weighted down in the stirred water bath until a few minutes before the final observation time. At first a microscope was arranged to record the cleavage time while the eggs were still under water in the bath, but poor optical definition caused more error in determining the end point than the second order temperature error of removing the Petri dish and its cover and making direct observation on the stage of the microscope in the room. The room temperature was usually only 1° to 3° C. above the bath temperature, and with practice it became possible to remove the moist chamber from the thermostat only about three minutes before taking the final count. Since the measure sought was the *difference* between the time to cleavage of the eggs in the experimental and control vessels, and as these were treated exactly alike, the error due to temperature rise at this time was considerably less than 1 per cent.

All glassware and solutions were brought to the temperature of the bath before use. The solutions of alcohol were made by volume, 2 per cent, 1½ per cent, 1 per cent, ½ per cent, ¼ per cent, ⅛ per cent, 1/16

per cent, 1/32 per cent, 1/64 per cent, thus covering a range of 128-fold Stronger solutions than 2 per cent were of no use for the present purpose as even 2 per cent inhibited cleavage. No correction was made for osmotic pressure since alcohol penetrates the cells readily, probably to greater concentration than in the sea water medium. Eggs were, in fact, worse affected if correction was made with distilled water.

Fairly heavy insemination of a sample of eggs was carried out in a dish at the bath temperature with immediate stirring to reduce the time for sperm-egg contacts. Eggs were then pipetted into the three experimental dishes, usually between 20 and 40 eggs being dispersed in each dish to avoid crowding or proximity. This number of eggs was found to be about as many as could be accurately counted at the end. Another sample of the same inseminated eggs was transferred to a Syracuse dish containing the experimental concentrations of alcohol, but not until 5 minutes after insemination. This period in sea water was kept to permit sperm entry to be well underway before any contact with alcohol, to rule out any effect of the alcohol upon the swimming or attachment of the sperm to the egg. The first alcoholic solution served as a wash bath to avoid dilution, and the eggs were at once placed about 20–40 each in the experimental dishes containing the alcoholic solutions. The moist chamber was then sealed and placed in the tank within a minute. The eggs were out of the bath longer at the start than at the finish, due to the 5-minute delay, but the temperature error was again second order and negligible.

When the Petri dish was removed from the bath and opened it fitted onto the stage of the microscope and all six dishes in it could be observed by rotating the Petri dish. As the eggs were being observed, counts were made of the exact number in each dish, and the time was counted when that egg divided which represented division of half of the eggs in the dish. This time was estimated when there were an odd number of eggs in the dish. It was found that with a certain type of illumination an optical property of the dividing egg could be used as a very sharp end point. As the two advancing cleavage furrows approached, the thin ectoplasm lining the furrows yielded a white glow, and the time at which the glowing films of the two approaching furrows touched could be determined within 15 seconds or less in a closely observed egg. Time was usually recorded to the nearest quarter minute for a given dish of eggs. Occasionally polyspermic or unfertilized eggs were present and these were eliminated from the count, but they were very rare. In no case at any time did the controls fertilize less than 99 per cent. *Urechis* eggs are exceedingly hardy and reliable when the animals are in good condition.

Results

The results are summarized in Table I. Figure 1 is a graph of the next to last column of Table I in which the horizontal axis (alcoholic concentration) is on a geometric progression scale to magnify space between the points at the left which would be almost superimposed on a linear scale.

TABLE I

Averaged results for each concentration of alcohol. The columns, left to right, show: (1) percentage of alcohol by volume, (2) total number of experiments in that percentage, each with a control, (3) average time from insemination until 50 per cent of the control eggs had cleaved, (4) average time until 50 per cent of the experimental eggs had cleaved, (5) the percentage increase in time lapse for experimental eggs, (6) the relative cleavage rate of the experimental eggs, counting the rate of the controls as 100 per cent, and (7) the range of percentages of the individual experiments entering into the average value given in 6.

Alcohol by volume	No. of experiment	Av. time controls	Av. time experimental	Increase time of experiment	Experimental relative rate, contr. = 100%	Range
per cent		*min.*	*min.*	*per cent*	*per cent*	*per cent*
2	9	153.2	∞	∞	0	0.0–0.0
1½	18	153.2	175.9	15	87	84.2– 91.6
1	15	152.4	157.8	3.5	96.6	95.4– 98.4
1/2	18	153.1	153.9	0.5	99.5	98.0–101.0
1/4	6	152.7	152.8	0.1	99.9	98.5–101.0
1/8	9	152.4	152.4	0	100.0	99.1–100.3
1/16	6	154.2	154.9	0.5	99.5	98.9–100.1
1/32	6	151.5	151.5	0	100.0	99.8–100.3
1/64	6	150.6	151.4	0.5	99.5	98.8–100.0

It was found that the time lapse to cleavage of the control eggs, taken day after day from the same female, steadily increased.[5] On April 2, 1932, the time was 148 minutes, while by April 16 it had become 157 minutes, an increase of 6 per cent in two weeks. Partly for this reason the measurements were staggered so that those in a given concentration of alcohol were made at various times over this period of steady shift. The significant measure is the ratio of the time lapse to cleavage of the experimental and the control eggs, and this is recorded in Table I by dividing the average time for all the experimental eggs

[5] Other more recent measurements using eggs from several different females recently collected suggest that the eggs from the female used in these experiments developed slightly more slowly than average. It is possible that the increased time lapse to cleavage may be due to slight infiltration of sea water into the segmental organs in which the eggs are stored once a pipette has been inserted into the gonopore as much as or more than to actual ageing or deficient nutrition of the female.

in a particular concentration of alcohol into the average time for the simultaneous controls.

In 2 per cent alcohol complete cleavages did not take place. Beginning usually about 170 to 175 minutes after insemination, *i.e.*, well after the controls had cleaved, the eggs in 2 per cent alcohol became irregular with both large and small protrusions or lobes. The lobing was probably aided by the lowering of surface tension by the alcohol.[6] An abortive attempt at cleavage was made. The nucleus usually divided but the cytoplasm did not. At about 195 minutes the eggs rounded up and became quiescent, bearing two nuclei which sometimes fused or came to lie closely together and sometimes remained well apart. At this time the controls were in the 4-cell stage. At about 230 minutes the eggs in 2 per cent alcohol again actively attempted cell division, with lobes and protrusions, but again abortively, with only the nuclei dividing. Cleavage attempts were thus both late and unsuccessful. Eggs which remained in 2 per cent alcohol later achieved some measure of incomplete cellulation, and after 24 hours had developed cilia which slowly rotated the abnormal larvæ. Eggs transferred from 2 per cent alcohol to fresh sea water just prior to attempts at cell division largely recovered. Polar body formation was normal in 2 per cent alcohol or less, although in alcoholic solutions made isosmotic by the addition of distilled water, the polar bodies were often very large, sometimes fused, and sometimes resorbed. Isosmotic solutions were not used in the experiments, as stated earlier, because of the ready penetration of alcohol into the cells.

In 1½ per cent alcohol some protrusions and irregularities accompanied cleavage, and the blastomeres were sometimes unequal, but a very large majority of the eggs ended up completely cleaved, with smooth blastomeres and normal nuclear distribution. The criterion chosen for counting the eggs just cleaved (described in the preceding section headed " Method ") was considerably less sharply defined, and in 1½ per cent alcohol it was necessary to estimate cleavage time more roughly. Cleavage was decidedly delayed in this concentration.

In 1 per cent alcohol the cleavage type was detectably altered. The optical glow of the ectoplasm lining the furrows was less sharply defined. There was a very slight tendency for the eggs to form protrusions. The cleavage type was very close to normal, however, in all respects.

In ½ per cent to 1/64 per cent alcohol the type of cleavage could not be distinguished from that of the normal controls.

[6] Pantin (13) has found that alcohol causes protrusions and pseudopods in a marine amœba.

Discussion

The *Urechis* egg, presumably with excess self-contained food, shows no stimulation of division rate, within less than 1 per cent, in sub-narcotizing concentrations of alcohol (Fig. 1). While it has not been directly proved that *Paramecium* and other Protozoa can use alcohol directly as a food, it is not improbable in view of its biochemical relations that alcohol can be utilized either directly or through the medium of microörganisms which may in turn be used as food. It is well estab-

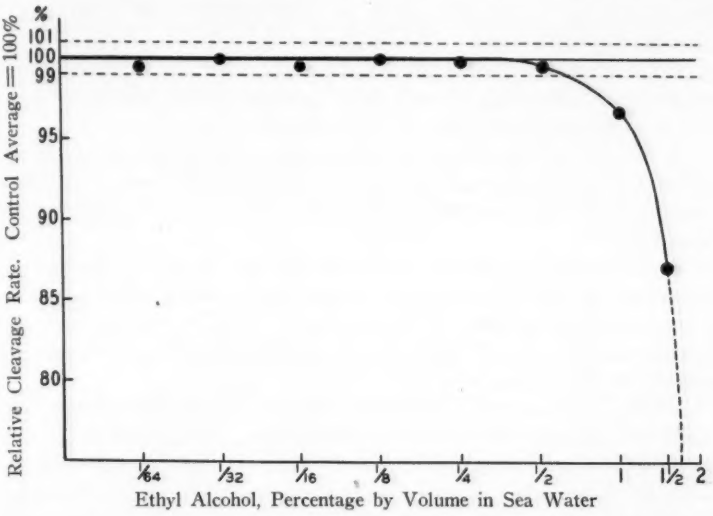

FIG. 1. The average cleavage rates (reciprocals of time lapse from insemination until 50 per cent of the eggs had just completed the first cleavage) of *Urechis* eggs in percentages of ethyl alcohol by volume in sea water. The rates are expressed as percentages of control rates. The horizontal axis is on a geometric progression scale to avoid superposition of points to the left. In 2 per cent alcohol (off scale) cytoplasmic division was inhibited.

lished that the food supply in the medium limits the rates both of growth and cell division in Protozoa, at least when the food supply is deficient. When there is an excess of food, other factors may limit the rate of cell division. If alcohol in sub-narcotizing concentrations is utilizable as a food, directly or indirectly, the different and apparently contradictory results in the protozoan studies might be due in part at least to this effect, with different effective nutrient content of the different media. From this point of view the lack of stimulation in the *Urechis* egg is not inconsistent with the results of the protozoan studies.

The *Urechis* egg in constant medium with self-contained food and not necessarily dependent upon growth[7] for cell division affords a comparatively simple and direct test of the effect of dilute alcohol on the rate of cell division.

Summary

1. Alcohol has no effect on the rate of cell division of *Urechis* eggs except to retard and inhibit. In 2 per cent alcohol the first cell division is inhibited, although the nucleus usually divides.

2. In 1½ per cent alcohol the time lapse to first cleavage is increased approximately 15 per cent. In 1 per cent alcohol it is increased approximately 3.5 per cent. (See Table I.)

3. A series of sub-narcotizing concentrations of alcohol covering the range ½ per cent to 1/64 per cent does not stimulate the rate of cell division nor affect it within limits of 1 per cent (see Fig. 1).

4. This covers the ranges in which stimulation to the extent of 30 per cent or more has in some cases been observed in Protozoa. In other cases, with the same and with other protozoan forms, no stimulation has been found.

5. It is suggested that the stimulation in the Protozoa, when it occurs, may be largely a nutritive phenomenon, where food supply is acting as a limiting factor to division rate. This involves the assumption that alcohol may be used as a food either directly or through the medium of microörganisms.

6. The *Urechis* egg, with self-contained food supply, presumably avoids this limiting factor. It has other points of simplicity for testing the effect of dilute alcohol upon cleavage rate, such as constant medium, which are discussed. The varying and apparently contradictory results of different investigators with the Protozoa and the results with *Urechis* eggs can be placed tentatively in agreement if certain assumptions are made.

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[7] See paragraph (3) under heading: Purpose of experiments here reported.

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FURTHER STUDIES OF EXOGASTRULATION IN THE SEA URCHIN

A. J. WATERMAN

(From the Department of Biology, Brooklyn College,[1] and the Marine Biological Laboratory, Woods Hole)

Introduction

Observation has shown (Herbst, 1893; MacArthur, 1924; Runnström, 1928; Driesch, 1893) that by means of a variety of external stimuli the processes involved in the gastrulation of the sea urchin could be inhibited at the fertilized egg stage and that the potential endoderm cells subsequently formed an exovation which differentiated into a tripartite gut. In these exogastrulæ the ectoderm generally differentiated the apical plate and stomodæum while the mesenchyme frequently underwent excessive proliferation to give accessory skeletal structures and various abnormalities of the skeleton. The agents which were found to produce these effects included LiCl, $CaCl_2$, diluted or stale sea water, $CuSO_4$, HgCl, KCN, all the lithium halides, HCl, sodium butyrate, and carbon monoxide.

In a previous study of exogastrulation in the sea urchin, *Paracentrotus lividus* (Waterman, 1932), it was found that hypotonic sea water, combinations of the isotonic solutions of the chlorides of Na, K, Mg and Ca, or $MgCl_2$ alone, inhibited gastrulation in many cases, disturbed the ecto-endoblastic determination of these embryos, and stimulated excessive mesenchyme proliferation at as late a stage of development as the blastula. The types and degree of exogastrulation were comparable with those secured by other methods, especially LiCl, in which the fertilized egg was used (MacArthur, 1924; Runnström, 1928). LiCl had the same effect upon the blastula stage as it had on the earlier developmental stage except that higher concentrations of the salt had to be used (*cf.* Herbst, 1893). Driesch (1893) had previously pointed out that an elevation of temperature to 30° during gastrulation inhibited invagination.

Since these experiments demonstrated that the differential susceptibility of the blastula stage differed but little from that of the fertilized egg, it has seemed desirable to repeat the experiments with the same

[1] Contribution No. 8.

stages of another species (*Arbacia punctulata*) and to use different methods. To this end various stages of blastulation in *Arbacia* were exposed to hypotonic sea water, lowering and elevation of temperature, and to X-rays for varying lengths of time, after which samples were transferred to fresh sea water. Thereafter daily examination of the cultures was made.

Experimental

Hypotonic Sea Water

In previous experiments in which blastulæ of the sea urchin *Paracentrotus lividus* were exposed to dilutions up to 55 per cent, it was found that 45 per cent and 50 per cent inhibited gastrulation in some individuals and resulted in the formation of shallow exogastrulæ. The exovations never developed to any extent and in certain cases there was observable only a constriction between ectodermal and endodermal regions. Dilution of sea water up to 20 per cent but slightly retarded development while dilutions of 55 per cent resulted in death and disintegration within a few hours. As higher dilutions were used, more and more of the blastulæ failed to invaginate and the weaker ones died.

In the present experiments spherical non-swimming blastulæ gave a few shallow exogastrulæ, total inhibition of any endodermal activity, or many apical constrictions in which the apical portion of the embryo was more or less separated from the remainder. In 60 per cent hypotonic sea water the number of exogastrulæ increased, while in 65 per cent dilutions many good cases were secured. Fusions occurred at 60 per cent but did not develop. Cases of the failure of the vegetal pole cells to do anything indicate that not only was gastrulation inhibited, but likewise the division mechanism of the cells. Embryos of this type are merely hollow balls of cells with the blastocœl more or less packed with large opaque cells.

The term " shallow exogastrula " is applied to the condition in which some endodermal outgrowth occurs but the exovate is frequently irregular, never of any considerable amount, and does not differentiate the three divisions characteristic of the gut. Subsequent development is limited to apical plate and stomodæum. No skeleton forms and these abnormalities do not long survive.

Early swimming blastulæ gave a few good cases of exogastrulation at 30 per cent dilution. The numbers increased up to 50 per cent and then fell off as higher dilutions of sea water were employed. Apical constrictions were common. As the more resistant individuals were affected by the higher dilutions, the weaker ones were killed and disintegrated.

Swimming oval blastulæ gave a few very simple exovates at dilutions of 55 to 60 per cent. Embryos which remained in 55 per cent hypotonic sea water developed both exovates and apical constrictions, while in 60 per cent excellent fusions, apical constrictions, and a few exovates appeared.

At a much later stage which shows the flattening of the vegetal pole, 65 per cent hypotonic sea water gave a very few cases of exogastrulation together with apical constrictions. Dilutions of 55 to 60 per cent gave a few typical constrictions only. Other abnormalities involved skeleton, gut, or arm formation.

An initial stage of gastrulation was also used to determine if the gastrulation process, once it was started, could be inhibited and this was followed by the external development of the endoderm. In no experiments with this stage were exogastrulæ found. Forty-five per cent dilutions gave no development beyond the triangular gastrula stage. Fifty per cent inhibited further gastrulations; 55 per cent resulted in no further development outside of a few apical constrictions; while 60 per cent caused death. At a more advanced stage of gastrulation 40 to 50 per cent dilutions gave stunted and abnormal plutei and 60 per cent gave apical constrictions but no development of arms. Retardation was in proportion to the dilutions employed.

Elevation of Temperature

Spherical, non-swimming blastulæ, exposed to a temperature of 28°–29° C. for 5 hours, continued their development during this period and when returned to normal room temperature (22.5° C.) were at the stage of flattening of the vegetal pole or initial gastrulation. No difference could be seen between them and the controls in regard to the degree of development. Twenty-four hours later most of the individuals of the culture had developed to the young pluteus stage, a relatively few had gastrulated but had remained in the globular form; in some gastrulation was abnormal, while about one per cent were definite but rather shallow exogastrulæ.

The same stage exposed to a temperature of 31–32° C. for 5 hours developed very slowly during this period. At the end of the 5 hours they were at the oval stage of blastulation and were swimming very slowly. Twenty-four hours later a few had died, most were at the triangular gastrula stage, and a few had gastrulated in an abnormal fashion. In addition there were present in the culture samples a few shallow exogastrulæ together with individuals in which the vegetal pole had ruptured giving a small mass of cells attached in this region. Forty-eight hours later the triangular gastrulæ had developed to the pluteus stage while the abnormalities had all died.

Older stages up to gastrulation were exposed to the same elevations of temperature. At 28–29° C. for 10½ hours the spherical swimming stage gave a few shallow exogastrulæ. At 31–32° C. young plutei developed after 24 hours at room temperature together with a few exogastrulæ. Many individuals had died. At 32–33° C. most of the individuals died after 24 hours in normal sea water. Gastrulation was entirely inhibited and only a few large opaque blastulæ with large swollen cells remained alive. At 33–34° C. all died.

The stage of initial gastrulation (flattening of the vegetal pole) kept at 30° C. for 1¼ hours gave mostly mature plutei with a few definite undifferentiated gastrulæ. At 32–33° C. for 1½ hours movement is slowed and 24 hours later many dead individuals were found in the cultures. The embryos either were unaffected or killed as no exogastrulæ were seen. A temperature of 35° C. for one hour killed all the embryos.

The stage of initial and earliest gastrulation was for the most part unaffected by a temperature of 30° C. for one hour. An occasional undifferentiated exogastrula occurred. At temperatures below 27° C. development appears to be unaffected.

For comparison eggs in the 2 to 4-cell stages were kept at a temperature of 30° C. for one hour. Most of these gave typical plutei in 24 hours and also an occasional shallow, irregular exogastrula. Eggs 30 minutes after fertilization exposed to 30° C. for 30 minutes developed many shallow exogastrulæ. At least 50 per cent of the embryos were abnormal in one way or another. The other stages studied were unaffected by an exposure of this strength and duration.

Lowering of Temperature

In another experiment samples from the same batch of eggs were transferred to the cold room at 2° C. at different stages of development and were kept at this temperature for varying lengths of time. Samples taken at 15, 30, and 45 minutes after fertilization, and of 2-cell, 4-cell, 8 to 16-cell, 32-cell plus, 64-cell and oval swimming blastula, were exposed to this temperature for 9 to 19 hours. Twenty-four to 48 hours after transfer to fresh sea water, stunted or abnormal plutei, globular embryos with gut and skeleton, gastrulæ, irregular gastrulæ, and blastulæ were found. A few shallow exogastrulæ occurred in the cultures of eggs which had been placed in the cold room 15 minutes after fertilization and at the 64-plus cell stage. Gastrulation was increasingly abnormal at the longer exposures, but in all cases development continued during the experiment. In some the irregular swimming blastulæ showed rupture of the vegetal pole. Oval swimming blastulæ were least affected, the younger stages more.

Swimming oval blastulæ were also exposed to 2°, 4°, and 7° C. for 24 hours. Temperatures of 2° and 4° C. gave an occasional simple exogastrula, but the majority were small plutei with a few blastulæ and abnormal gastrulæ. Young non-swimming blastulæ were exposed to temperatures of 2°, 4°, and 7° C. Five hours at 2° C. gave mature plutei together with a few globular individuals in which the gastrulation process was entirely inhibited. Gastrulation occurred during the experiment.

Similar stages kept at a temperature of 4° C. for 18 hours gave small plutei and an occasional shallow exogastrula or abnormal gastrulation. During sojourn at 7° C. for 18 hours development progressed as far as the oval, swimming blastula stage. At this time the temperature was slowly raised to 31–32° C. and maintained for 9 hours. Twenty-four hours later most of the embryos were dead without having gastrulated. A few irregular gastrulæ showed lethargic movement. Each one of these two environmental changes alone is not sufficient to affect the process of gastrulation to any significant extent, but when combined effectively prevent it.

From this data it seems evident that elevation or lowering of temperature has little influence upon the factor or factors responsible for gastrulation except, in the very weakest individuals, to make the process somewhat irregular. An occasional exogastrula may develop around 2–4° C., or gastrulation may be entirely inhibited.

X-Ray

A few preliminary experiments were also made in which both fertilized eggs (25 minutes after fertilization) and blastulæ (spherical and oval swimming embryos) were exposed to 720 revolutions per minute at 20 cm. distance for the same lengths of time, *i.e.*, 2 to 12 minutes. Samples were removed at 2-minute intervals.

In agreement with the results described above, it was found that both eggs and blastulæ vary markedly in their susceptibility, but there is little correlation between length of exposure and the amount or degree of effect as regards gastrulation. In both cases apparently normal although somewhat smaller plutei were found even after 12 minutes' exposure to the stimulus. With increasing length of exposure, more of the weaker individuals were killed and fewer others reached the pluteus stage.

Fertilized eggs exposed for 2 minutes develop blastulæ to young plutei in 48 hours. These die much earlier than the controls, indicating that their resistance has been weakened by the treatment, as normal

plutei will live for days before dying of starvation. The number of plutei appearing in the cultures steadily decreases following progressively longer exposures and more individuals fail to gastrulate or never get beyond the gastrula stage. In those cases where the gastrulation process has been inhibited, the endoderm shows very little growth. Some outpushing of the endodermal cells does occur but it is very slight.

Thus not only has there occurred an inhibition of the gastrulation process but also an interference with the division rate of the cells. In other cases where gastrulation has occurred the endoderm has been affected in that it is present as large or small irregular masses of cells near the vegetal pole. The gut is very abnormal. An occasional exogastrula occurs in cultures exposed from 6 to 12 minutes but the exovation is very shallow and shows no differentiation. Other abnormalities noticed include apical constrictions and rupture of the vegetal pole similar to those described above, and development of a few giant transparent circular blastulæ lacking most of the mesoderm.

As is the case with the fertilized eggs, the effect upon the blastulæ is to retard and inhibit further development in the weaker individuals. In cultures exposed from 2 to 12 minutes more and more of the weaker individuals are affected depending upon their resistance. Effects vary all the way from death, inhibition or retardation of gastrulation, to modification of the apical portion of the body skeleton. The body rods frequently are sharply pointed. The blastula stage seems to be as susceptible as the fertilized egg stage.

A further abnormality concerned those individuals which had failed to escape entirely from the vitelline membrane. Such individuals are constricted by the partially enclosing membrane into double formations comparable to exovations, but they generally fail to gastrulate. The possible effect of the X-rays is to harden the membrane to such an extent that the enzyme produced by the embryo is not capable of destroying it, and the embryo is unable to escape entirely from the rigid shell. In cases where escape has been successful the empty membranes may persist for some time.

Embryos at the stage of the flattening of the vegetal pole (initial gastrulation) are much more resistant to X-rays than the stages just described. Cultures of this stage exposed to similar intensities and for the same length of time show fewer deaths, a higher percentage of normal plutei, and almost 100 per cent gastrulation. Only occasionally was gastrulation completely inhibited and this inhibition also involved any endodermal proliferation.

Discussion

The methods used in the present experiments serve not only to increase the list of those stimuli which have been shown to cause inhibition of the gastrulation processes and the external development of endoderm, but also indicate that stimuli other than chemical ones may produce a similar effect although not to the same degree. Fewer cases of exogastrulation were secured in these experiments than in those previously reported for *Paracentrotus lividus,* and, moreover, very few of them underwent any further differentiation. This may indicate that either the gastrulation processes in *Arbacia* are less susceptible to environmental stimuli than in *Paracentrotus,* or the stimuli employed affected other developmental processes more adversely.

In general, the results of these and previous experiments point to a high degree of susceptibility of the gastrulation processes to changes in the external environment throughout early development. If a comparison is made of the types of exogastrulæ and their subsequent differentiation between those secured following exposure at the fertilized egg stage and those at the blastula stage in *Paracentrotus* alone, it is seen that more typical differentiation of larval organs follows the exposure of the earlier stages. In the older stages subsequent development is more abnormal and retarded. Thus, while more cases appear with the earliest stage and they show more complete subordinate differentiation, with the older stages fewer cases are secured and these are more atypical as to ectodermal and skeletal differentiations. The gastrulation processes become increasingly more difficult to disturb, while on the other hand histogenic processes are more easily affected.

Various ideas have been advanced for the explanation of exogastrulation. Some have referred to a differential growth process (Herbst, 1893; Driesch, 1893), while others have been mechanical in nature, as for instance: (*a*) differences in rate of growth between ectodermal and endodermal portions of blastula whereby a lateral pressure is exerted on the ectodermal plate; (*b*) resistance of the closely pressed egg membrane, so that the surface increase of the blastula is possible only internally; (*c*) constantly decreasing amount of fluid in the blastocœl perhaps exerts a sucking effect on the endodermal plate; and (*d*) the decreased ectodermal area (Lithium effect) is unable to accommodate the enlarged archenteron in its interior (Huxley and DeBeer, 1934, p. 334). From the available data and also from the fact that fertilized eggs, cleavage stages, and blastulæ will give exogastrulæ, it would appear that any mechanical explanation of this phenomenon is inadequate. All of these are not free of criticism since the membrane is separated from the blas-

tula by the perivitelline space filled with fluid and the fluid within the blastocœl would have to be displaced, and further since the membrane disappears from the blastula stage of both *Paracentrotus* and *Arbacia* early in blastulation.

Recently Moore (1930) has found that an excess of Ca added to sea water causes displacement of the nuclei of *Strongylocentrotus purpuratus* blastulæ to the inside, while Li-larvæ of *Dendraster* and *Strongylocentrotus* showed nuclei in the periphery. From this he reasons that nuclear position does not correspond to invagination or evagination. In cell plates from fragmented larvæ of *Dendraster* the cell chains are bent or curved; accordingly he suggests that invagination is caused by asymmetrically placed cell bridges. If this is true, then the placement of these cell bridges can be affected not only at the fertilized egg stage before any cleavage has occurred, but also during any stage of cleavage or blastulation up to initial gastrulation when the cell bridges are already established.

Runnström does not explain the failure of the endodermal cells to invaginate while MacArthur appeals to such mechanical factors as pressure within the cavity of the blastocœl and lack of space due to the large amount of mesoderm produced by the inhibiting action of LiCl. On the whole, the cause of the invagination of the cells of the vegetal pole of the blastula is but very imperfectly understood. Consideration of the great variety of stimuli which may inhibit gastrulation and the fact that this inhibition may occur at different developmental stages seems to indicate that it is a question of an action upon a specific potentiality localized at the vegetal pole of the egg and blastula stages.

During cleavage this potentiality is segregated in the macromeres at the vegetal pole, endowing these cells with the capacity to push into the cavity of the blastocœl. Thus any agent which would act to inhibit this property in the fertilized egg would likewise affect the cells containing it at the blastula stage.

This idea of a specificity of the vegetal region is not a new one, as Hörstadius (1927) has been able to demonstrate by means of vital coloration and microdissection that one of the determining factors in the type of segmentation in *Paracentrotus* is the existence of a particular kind of autoplasm in the vegetative hemisphere of the egg (micromeres and macromeres) which acts in the capacity of an organizer upon the presumptive ectoderm. This author has also shown by a study of the developmental potencies of pieces smaller than halves, that there exists in the early embryo a distribution of potencies along the primary egg axis. At the 64-cell stage the lower group of macromeres represents

potential endoderm while the micromeres contain the presumptive primary mesenchyme.

The numerous stimuli which have been found to inhibit gastrulation at the blastula stage of development act upon certain potencies of the presumptive endodermal cells in such a manner as to prevent invagination. Accordingly, the endoderm differentiates as an exovate. The gastrulation process is very sensitive to environmental changes at any stage of early development but becomes increasingly more difficult to disturb in progressively older stages. Furthermore, its susceptibility varies in different individuals of the same culture and also with the kind of stimulus. The micromeres are also affected since the mesenchyme generally undergoes precocious proliferation.

Summary

1. The phenomenon of exogastrulation characterized by inhibition of the gastrulation processes, external differentiation of endoderm, and the excessive proliferation of mesenchyme, has been produced in *Arbacia punctulata* by exposure of blastula stages to hypotonic sea water, lowering and elevation of temperature, and X-rays.

2. Dilutions of sea water of 45 to 65 per cent give the most numerous cases of the various methods employed. A few occur at 30 to 40 per cent dilutions.

3. Development is typical at 27° C. but at higher temperatures becomes increasingly irregular and abnormal. A temperature of 35° C. kills the cultures or if exposure is for a short time only, inhibits any further differentiation. Shallow exogastrulæ appear at 28° to 31° C.

4. Lowering of temperature has little effect upon gastrulation. Development is fairly typical but retarded at 7° C. At 2° to 4° C. an occasional exogastrula occurs while the stronger individuals eventually gastrulate at this temperature.

5. In the X-ray experiments, dosages of 720 revolutions per minute at 20 cm. distance for 6 to 12 minutes give a few exogastrulæ.

6. Results indicate that stimuli other than chemical ones will inhibit gastrulation at the blastula stage of *Arbacia*. Great injurious effect on subsequent development is apparent since the exogastrulæ fail to differentiate except for apical plate and stomodæum. The exovation is usually shallow.

7. Fusions, ectodermal constrictions, and permanent blastulæ are common even where no exogastrulæ appear.

8. With increase in length of exposure and in strength of stimulus, the more resistant individuals are affected while the weaker ones are killed or fail to show any further development. Various stages of blastulation appear equally sensitive.

9. Examination of the long list of stimuli which will cause exogastrulation would seem to show that any mechanical explanation is unsatisfactory since this phenomenon can be provoked by exposing fertilized eggs, cleavage and blastula stages. It is concluded that some potentiality present in the vegetal pole of the fertilized egg and later segregated in the vegetal cells (presumptive endoderm) of the blastula causes the inturning of cells during gastrulation. This is very sensitive to environmental changes at any stage of development up to the time of gastrulation but varies in susceptibility in different individuals. Accompanying phenomena of cell division and differentiation are also affected according to the stimulus employed and length of exposure.

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THE LIFE HISTORY OF THE COPEPOD TORTANUS DISCAUDATUS (THOMPSON AND SCOTT)

MARTIN W. JOHNSON

(From the University of Washington Oceanographic Laboratories, Seattle, Washington)

Introduction

During the examination of a series of plankton samples taken at Friday Harbor, Washington, over a period of about four years (1924, 1926–1929), an undescribed microscopic egg was encountered in rather small numbers but with such regularity throughout the warmer months that a record was kept of its occurrence. This record and a brief description of the egg were included in the report on the plankton investigation (Johnson, 1932). Shortly before the investigation was terminated, a number of these eggs were taken from the plankton and hatched in culture dishes in the laboratory at Friday Harbor. The hatched larva proved to be an undescribed copepod nauplius. However, it did not survive beyond the first stage and my removal to the East Coast prevented further experimentation at that time.

The work was again resumed in early September, 1933, and as a result of the study the egg was determined to be that of the planktonic copepod *Tortanus discaudatus* (Thompson and Scott). Due to the increased recognition of the importance of studying the developmental stages of animals occurring in the plankton, the successive nauplius and copepodid stages were followed through and are here described.

Tortanus discaudatus is a common neritic copepod with a wide distribution in boreal waters of the Pacific and Atlantic coasts of North America. Seldom does it occur in very large numbers, but it may assume an important rôle in neritic communities. Willey (1920) reports that it composes 50–75 per cent of the summer copepod plankton off Souris, Prince Edward Island, and in Passamaquoddy Bay at St. Andrews, New Brunswick, it was found at times to constitute over 40 per cent of the total animal community of the plankton in the warm months (Fish and Johnson, unpublished data). It is the only species of this genus recorded from Puget Sound waters.

The author wishes to thank Dr. T. G. Thompson, Director of the University of Washington Oceanographic Laboratories, for laboratory facilities and Dr. C. B. Wilson for final criticism and suggestions.

Method

Eggs were removed from fresh plankton hauls and placed in filtered sea water for the culture of the first nauplius stage. This stage was also hatched from eggs which had been deposited in culture dishes by adults taken from the plankton. The eggs could not be obtained in large numbers and were therefore usually kept in watch glasses that could be used directly under the microscope. Adults and older larvæ were cultured in finger bowls or section jars. An even temperature was maintained by surrounding the dishes with cold running water. All later larval stages used in the study were obtained directly from the plankton. Mixed diatoms were placed in the culture dishes but there was no evidence of the larvæ having fed upon them. If cultures of tintinnids had been available for feeding, better results in rearing might have followed, for there is evidence that these ciliates constitute a large item in the diet of the larvæ. Adults were fed copepod larvæ taken from the plankton.

All drawings were made with the aid of a camera lucida.

Procedure and Results

The pelagic eggs removed from the plankton and cultured in the laboratory never failed to hatch within three days. Repeated attempts gave the same result, of which the following is typical:

September 11. Ten eggs placed in culture dish.
" 12. One egg hatched.
" 13. Five additional eggs hatched.
" 14. Four additional eggs hatched.

All the larvæ hatched from these eggs lived less than four days, none surviving to pass into the second nauplius stage. The five following nauplius stages could, however, readily be recognized and selected from the general plankton material for there is no other copepod nauplius with which it might easily be confused in this region.

The metamorphosis occurring at the critical moult between the sixth nauplius and first copepodid stages results in such drastic changes in the structure that it is not possible to bridge this gap by observations on added spines, segmentation, etc., as is practical in the individual successive stages of either the nauplii or copepodids. Oberg (1906) overcame this difficulty by removing larvæ from the plankton and rearing them experimentally through the critical moult. In the present species the general shape of the body, the color of the second antennæ, and the structure of the second maxillæ of the last nauplius stage were valuable

hints that suggested a relationship to the first copepodid stage of *Tortanus discaudatus* which was recognized in the plankton tows. These hints, together with the fact that the eggs and larvæ of this species were as yet unknown, led to an attempt to obtain eggs definitely known to have been spawned by *Tortanus discaudatus*.

On October 18 ten female and eight male adults were taken from the plankton and placed in culture dishes in the laboratory. At least two of the females were obviously ripe and had spermatophores attached when they were placed in the dish. On the following day it was found that 73 eggs had been deposited in several groups of double rows as shown in Plate I, Fig. 1. The greatest number in a group was 20. Later development of the egg case showed it to be like that of the pelagic egg taken from the plankton. Only a few of the eggs made much progress in development. However, four embryos did develop, and two of these hatched to normal nauplius larvæ identical with the first nauplius resulting from the pelagic egg of the plankton. The remaining two, although active and apparently normal, were unable to escape from the case enclosing them. The natural opening of the case was doubtless prevented by imperfections in its formation. This will be more fully discussed later.

The Egg

(Plate I, Figs. 3 and 4)

The egg proper is 0.11 mm. in diameter and is contained in a case or capsule especially constructed for flotation by the development of a thin, flat disc surrounding the equator of the single egg. This disc is 0.08 mm. in width, making the whole case 0.27 mm. in width as seen from above. The egg is granular and opaque due to the large amount of deuteroplasm, and the case is semi-transparent with very fine reticulations on the disc. One side of the case is provided with a fissure which serves as an operculum to open for the escape of the larva (Plate I, Figs. 3 and 4, *o*).

When the eggs are first deposited they are spherical and the specialized flotation case so characteristic of the older eggs found in the plankton is wanting. This disc-like case is formed some time after the eggs have been cast into the water. Its early formation is shown in Plate I, Figs. 1 and 2. The first indication of its development is seen in the appearance of a number of small blister-like structures covering the egg's surface. Finally one side (apparently predetermined, as suggested by the orientation of the eggs shown in Plate I, Fig. 1) is drawn out, forming a thin plate of double thickness. This plate spreads laterally until it results in the disc surrounding the egg at the equator.

Most of the eggs deposited in the culture dish showed some progress in the development of the case but none were so completely and symmetrically formed as were the cases enclosing eggs taken directly from the plankton. One feasible explanation for the failure to form perfect cases in the cultured material is found in the fact that the freshly spawned eggs are slightly mucilaginous and therefore adhere lightly to the bottom of the culture dish. This adherence would interfere with the proper expansion of the cases. Interference of this kind would be absent in the natural planktonic environment where the spawning is directly into deep water and where the membranes have an opportunity to shape and harden while the egg is still in suspension.

The Nauplius Larvæ

In the living condition the nauplius larvæ are readily recognized by the conspicuous orange-red pigment in the outer part of the relatively long second antennæ. This coloration is present in all the nauplius stages but has a tendency to become somewhat darker as the older stages are reached. The pigmentation is also present in the mandibles though less conspicuous. The eye is dark red. Other characteristics common to the nauplius stages are: (1) the presence of a large broad labrum which is narrowed at the anterior end and indented on the posterior margin which, following the first stage, is fringed with long weak bristles, (2) the colorless, long and slender anterior antennæ, (3) the hind part of the body being straight rather than flexed ventrally as in the nauplius of some species, and (4) the anterior end of the body being angular in outline in all but the first stage.

Stage I (Plate I, Fig. 5)

Body 0.12–0.14 mm. long, dorsal aspect egg-shaped with broader anterior end; posterior end with two medium strong, slightly curved spines of equal length.

First antenna. Three segments, the first fused with the second and bearing one short ventral bristle, the second with one long ventral bristle, and the third or distal with three long terminal bristles.

Second antenna. Coxopodite with one masticatory hook. Basipodite with one very small masticatory hook. Endopodite of one segment with one lateral and two terminal bristles. Exopodite of six segments: the first completely fused with the second and with no bristle, 2–5 with one long bristle each, and the sixth with one long and one shorter bristle.

Mandible. Coxopodite with an inconspicuous blade-like process bearing a very small spine. Basipodite with two small spines on the

inner side. Endopodite of two fused segments discernible only by the armature; the first segment with two short bristles and the second with three longer bristles. Exopodite of four segments, 1–3 with one bristle each, the fourth with two bristles.

Stage II (Plate I, Fig. 6)

Body 0.185–0.205 mm. long, slightly angular anteriorly, posterior end armed with one long plumose bristle and one shorter weaker plumose bristle situated at its left; the shorter (left) bristle curved ventrally. Laterad to each of these bristles is a series of very minute bristles.

First antenna. As in I, but the second segment with one long and one short ventral bristle, the distal segment with three long bristles and one shorter weaker accessory bristle at the tip.

Second antenna. Coxopodite with one very strong masticatory hook. Basipodite with one strong masticatory hook nearly as long as that on the coxopodite but more slender, and one weak short bristle on the posterior margin near the outer end. Endopodite of one segment with three long terminal and two shorter lateral bristles. Exopodite of six segments, the first fused with the second and bearing no bristle, the second with one long and one short bristle, 3–5 with one long bristle each, the sixth with one long and one short bristle.

Mandible. Coxopodite as in I, but with spine more pronounced. Basipodite with three spines on inner side. Endopodite segments as in I, the first with a strong hook and two bristles directed inwardly, the second with four posteriorly directed bristles. Exopodite as in I, but with one long and one short bristle on the first segment.

Stage III (Plate I, Fig. 7)

Body 0.23–0.26 mm. long, the posterior end bearing two long terminal spines and between them two longer plumose bristles (the left

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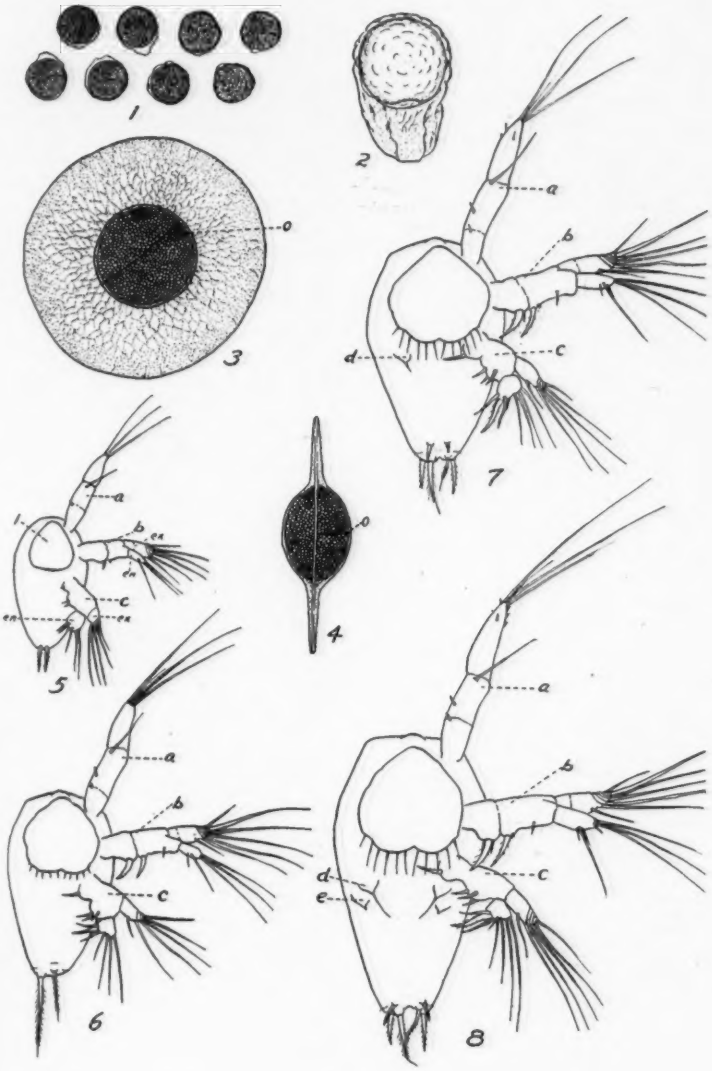
Abbreviations: *a*, first antenna; *b*, second antenna; *c*, mandible; *d*, first maxilla; *e*, second maxilla; *en*, endopodite; *ex*, exopodite; *f*, maxilliped; *g*, first feet; *h*, second feet; *i*, third feet; *l*, labrum; *o*, fissure.

Explanation of Plate I

1. Eggs as deposited.
2. Egg showing formation of case.
3. Egg and completely formed case, viewed from above.
4. Egg and case viewed from side.
5. Nauplius, stage I.
6. Nauplius, stage II.
7. Nauplius, stage III.
8. Nauplius, stage IV.

PLATE I



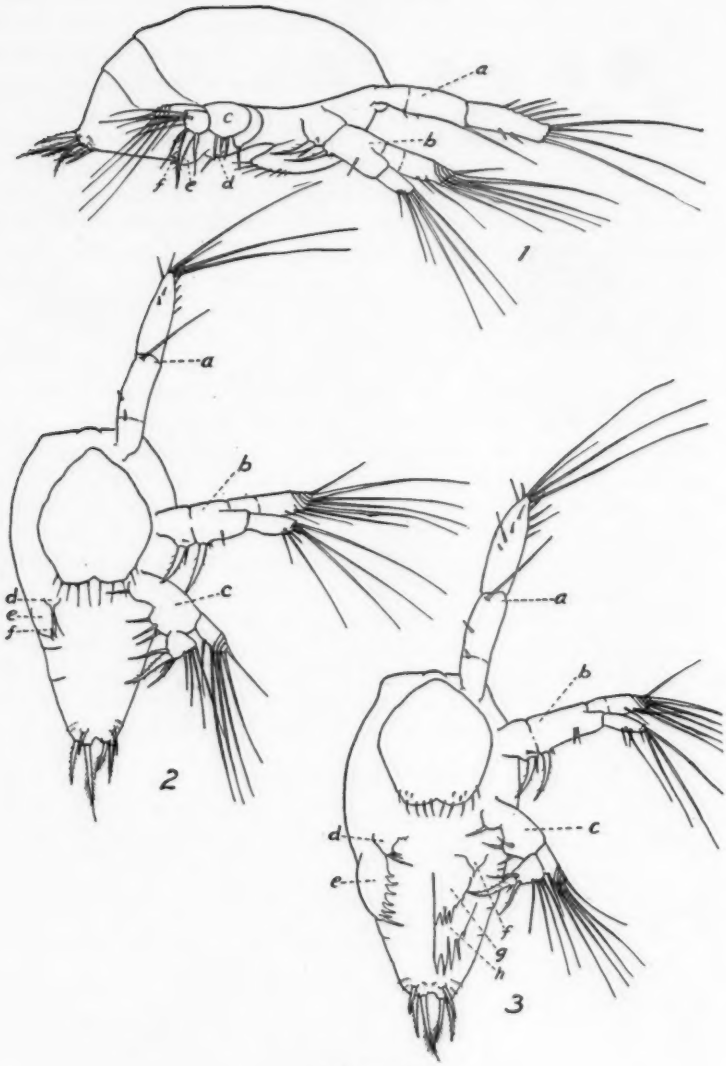


PLATE II

1. Nauplius, stage V, viewed from side.
2. Nauplius, stage V.
3. Nauplius, stage VI.

being slightly ventral), and ventrally a pair of ventral hooks. Minute bristles present as in II.

First antenna. As in II, but with two short dorsal marginal bristles and one short ventral marginal bristle on the distal segment.

Second antenna. Coxopodite with one very strong masticatory hook and one small spine situated immediately beside it. Basipodite as in II. Endopodite as in II, but with one long and two short lateral bristles. Exopodite as in II.

Mandible. All parts as in II, but with an additional small bristle on the first segment of the endopodite.

First maxilla. Indicated only as a bud with a very weak bristle.

Stage IV (Plate I, Fig. 8)

Body 0.30 mm. long, the posterior end somewhat elongated, furcal armature as in III.

First antenna. As in III, but with four short dorsal marginal bristles and one short ventral marginal bristle on the distal segment.

Second antenna. Coxopodite and basipodite as in III. Endopodite as in III, but with the addition of one short terminal bristle. Exopodite as in III.

Mandible. All parts as in III, but with increased strength of spines.

First maxilla. As in III.

Second maxilla. Indicated only as a bud.

Stage V (Plate II, Figs. 1 and 2)

Body 0.32–0.34 mm. long, posterior end armed as in IV.

First antenna. As in IV, but with six short dorsal marginal and two short ventral marginal bristles on the distal segment.

Second antenna. Coxopodite, basipodite, and endopodite as in IV. Exopodite as in IV, but with one long and two shorter weak bristles on the second segment.

Mandible. All parts as in IV.

First maxilla. As in IV.

Second maxilla. Indicated as a bud.

Maxilliped. Indicated as a bud.

Stage VI (Plate II, Fig. 3)

Body 0.26 mm. long, posterior end armed as in V.

First antenna. As in V, but with six short dorsal marginal and four short ventral marginal bristles on the distal segment. Through the successive stages the distal segment has become relatively longer so it now about equals in length the other two combined.

Second antenna. Coxopodite as in V. Basipodite as in V, but with two short weak bristles placed side by side on the posterior margin near the outer end. Endopodite and exopodite as in V.

Mandible. As in V.

First maxilla. With three small unequal terminal bristles and two inconspicuous spines at its base.

Second maxilla. Rudimentary, with seven weakly chitinized bristles.

Maxilliped. Indicated only as a bud.

First and second feet. Rudimentary.

Copepodid Stages

Stage I (Plate III, Figs. 1 and 2)

Body 0.55 mm. long, thorax of four segments, urosome of one deeply constricted segment. Caudal rami symmetrical, each with three long terminal bristles, one short dorsal bristle, and two outer lateral spines. In life the first antennæ are noticeably directed upward as well as laterally and the urosome is also flexed strongly upward. The second antennæ are deep reddish-brown. This pigmentation may occur as isolated patches near the base of the appendages and is also found on the ventral side of the thorax. The coloration persists in the later stages but is less obvious in the second antennæ.

First antenna. Eight segments.

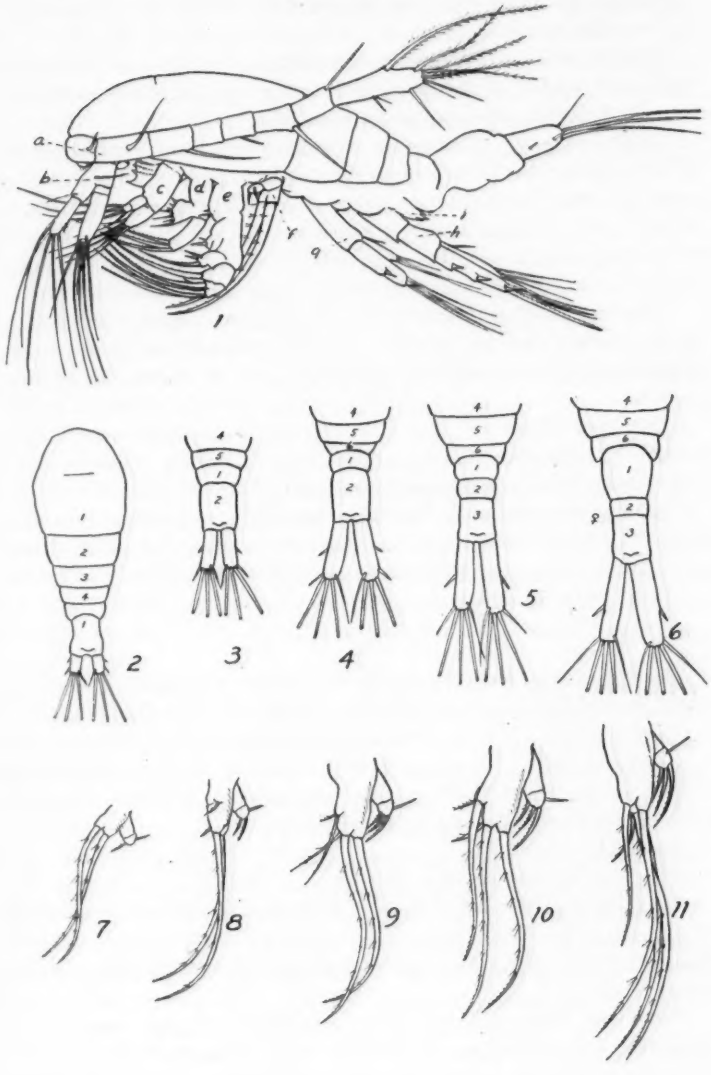
Second antenna. Coxopodite a short segment with no hook. Basipodite with one posteriorly directed bristle. Endopodite of two segments, the first being fused with the basipodite and bearing one distal bristle. The second segment bears at the tip five long bristles and on the inner margin a series of short fine bristles. Exopodite of four or five segments, the first short and with no bristles, the second long and bearing two long bristles, 3–4 very short with a total of four long bristles.

Mandible. Mandibular blade strong and provided with three well-defined teeth and one or two smaller teeth. The palp consists of a short

Explanation of Plate III

1. Copepodid, stage I, viewed from side.
2. Copepodid, stage I, dorsal outline.
3. Urosome and caudal rami, stage II.
4. Urosome and caudal rami, stage III.
5. Urosome and caudal rami, stage IV.
6. Urosome and caudal rami, stage V. [Segments of thorax and urosome as numbered.]
7. Maxilliped, stage I.
8. Maxilliped, stage II.
9. Maxilliped, stage III.
10. Maxilliped, stage IV.
11. Maxilliped, stage V.

PLATE III



coxopodite, long basipodite, and two short rami, the inner having two segments and bearing four long bristles at the end, the outer ramus bearing five long bristles.

First maxilla. There is a short rounded inner lobe armed with eight short bristles of unequal length, and a long outer segment bearing three long, strong, coarsely plumose bristles.

Second maxilla. Uniramose, very long and strong and composed of six or seven poorly defined wedge-shaped segments. The distal segments are armed with six long, strong coarsely plumose bristles, and one short coarsely plumose bristle. There are also six short bristles on the inner margin of the proximal segments. Two of these bristles are situated at the base of the first long bristle (Plate III, Fig. 1, *e*).

Maxilliped (Plate III, Fig. 7). First basal armed with two long, strong coarsely plumose bristles. Inner branch with one short plumose bristle directed anteriorly and one short smooth bristle directed posteriorly.

First feet (Plate IV, Fig. 1). Coxopodite and basipodite with no bristles. Endopodite unsegmented with seven bristles. Exopodite unsegmented with two outer spines and four bristles.

Second feet (Plate IV, Fig. 6). Coxopodite and basipodite with no bristles. Endopodite unsegmented with six bristles. Exopodite unsegmented with three outer spines, a terminal blade, and three inner bristles.

Third feet. Rudimentary.

Stage II

Body 0.74 mm. long, thorax of five segments, urosome of two segments, caudal rami symmetrical as in I (Plate III, Fig. 3).

First antenna. Eleven or twelve segments.

Second antenna. Coxopodite and basipodite as in I. Endopodite as in I, but with one short and four long terminal bristles. Exopodite as in I but with a total of only five bristles.

Mandible. As in I.

First and second maxilla. As in I.

Maxilliped (Plate III, Fig. 8). First basal with two long bristles as in I, and two very short bristles. Inner branch as in I, but with two plumose anteriorly directed bristles and one smooth posteriorly directed bristle.

First feet (Plate IV, Fig. 2). Coxopodite with one inner bristle. Basipodite with no bristle. Endopodite of two segments, the first with one inner bristle and the second with seven bristles. Exopodite of two segments, the terminal one with two outer spines and five end and inner bristles.

Second feet (Plate IV, Fig. 7). Coxopodite with one inner bristle. Basipodite with no bristle. Endopodite unsegmented, with eight bristles. Exopodite of two segments, the first with one outer spine; the second with two outer spines, a terminal blade, and four inner bristles.

Third feet (Plate IV, Fig. 11). Coxopodite and basipodite with no bristles. Endopodite unsegmented, with six bristles. Exopodite unsegmented, with three outer spines, a terminal blade, and three inner bristles.

Fourth feet. Rudimentary.

Stage III

Body 0.96 mm. long, thorax of six segments, urosome of two segments, caudal rami symmetrical as in II (Plate III, Fig. 4).

First antenna. Fourteen segments.

Second antenna. As in II, but with a group of minute inner bristles on the distal end of the first segment of the endopodite.

Mandible. As in II.

First maxilla. As in II.

Second maxilla. As in II, but with seven long, strong bristles and one short bristle on the distal segments and one small claw-like bristle on the basal segment.

Maxilliped (Plate III, Fig. 9). First basal with two long bristles as in II and with three short bristles. Inner branch as in II, but with three plumose anteriorly directed bristles and one smooth posteriorly directed bristle.

First feet (Plate IV, Fig. 3). Coxopodite with one inner bristle. Basipodite with no bristle. Endopodite of two segments, the first with one bristle and the second with eight bristles. Exopodite of two segments, the first with one small inner bristle and the second with two outer spines and five end and inner bristles.

Second feet (Plate IV, Fig. 8). Coxopodite with one inner bristle. Basipodite with no bristle. Endopodite unsegmented, with nine bristles. Exopodite of two segments, the first with one outer spine and the second with three outer spines, a terminal blade, and five inner bristles.

Third feet (Plate IV, Fig. 12). Coxopodite with one inner bristle. Basipodite with no bristle. Endopodite unsegmented, with nine bristles. Exopodite of two segments, the first with one outer spine; the second with two outer spines, a terminal blade, and four inner bristles.

Fourth feet (Plate IV, Fig. 15). Coxopodite and basipodite with no bristles. Endopodite unsegmented, with six bristles. Exopodite unsegmented, with three outer spines, a terminal blade, and three inner bristles.

Fifth feet. Rudimentary.

Stage IV

Body 1.11 mm. long, thorax of six segments, urosome of three segments in both male and female, caudal rami asymmetrical, the right ramus being slightly the longer (Plate III, Fig. 5).

First antenna. Sixteen segments.

Second antenna. As in III.

Mandible. As in III.

First and second maxilla. As in III.

Maxilliped (Plate II, Fig. 10). First basal with three long, strong bristles and two short bristles. Inner branch as in III.

First feet (Plate IV, Fig. 4). Coxopodite with one inner bristle. Basipodite with no bristle. Endopodite and exopodite as in III.

Second feet (Plate IV, Fig. 9). Coxopodite with one inner bristle. Basipodite with no bristle. Endopodite unsegmented, with ten bristles. Exopodite of two segments, the first with one outer spine and one inner bristle; the second with three outer spines, a terminal blade, and five inner bristles.

Third feet (Plate IV, Fig. 13). Coxopodite with one inner bristle. Basipodite with no bristle. Endopodite unsegmented, with eight bristles. Exopodite of two segments, the first with one outer spine; the second with three outer spines, a terminal blade, and five inner bristles.

Fourth feet (Plate IV, Fig. 16). Coxopodite with one inner bristle. Basipodite with no bristle. Endopodite unsegmented, with eight bristles. Exopodite of two segments, the first with one outer spine; the second with three outer spines, a terminal blade, and five inner bristles.

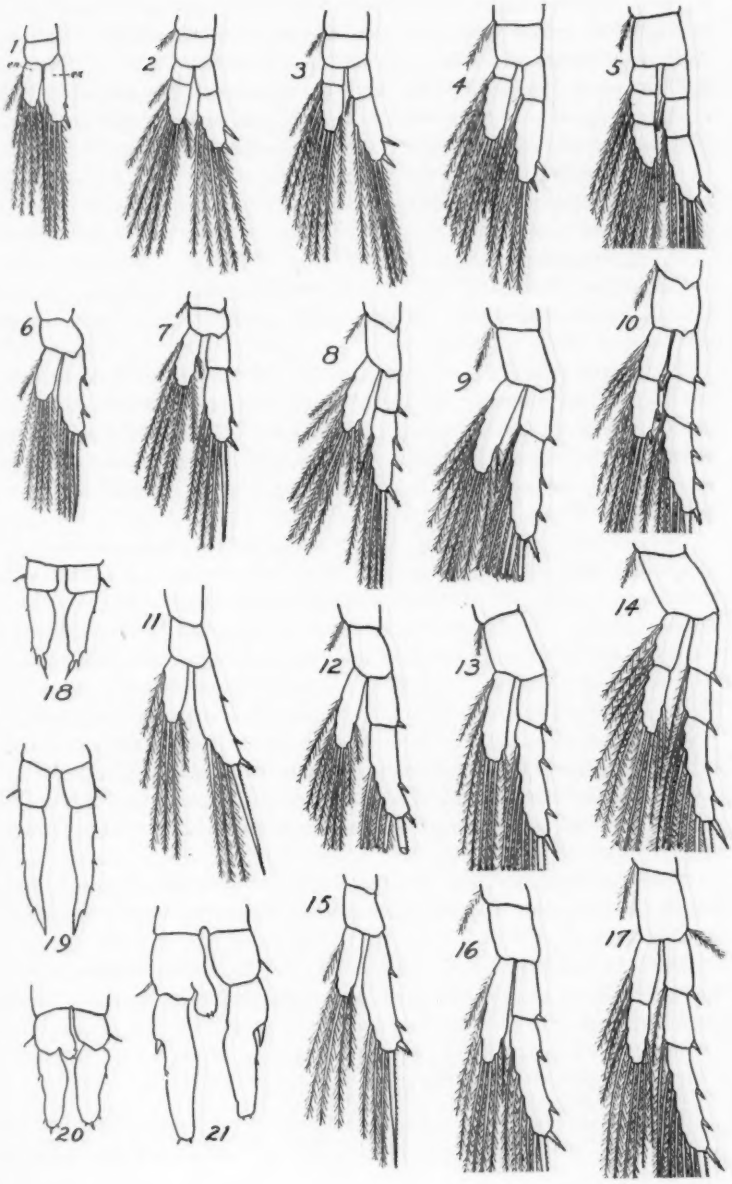
Fifth feet female (Plate IV, Fig. 18). Uniramose, symmetrical, of two segments, the first with one outer bristle, the second with two small outer marginal spines and one small and one larger terminal spine.

Fifth feet male (Plate IV, Fig. 20). Uniramose, asymmetrical, of two segments. The first segment of right foot with one short outer bristle and an inner thumb-like process bearing two small spines, the second segment with one small outer marginal spine and three terminal spines. Left foot as the right but wanting the thumb-like process.

EXPLANATION OF PLATE IV

1–5. Development of first feet, stages I–V.
6–10. Development of second feet, stages I–V.
11–14. Development of third feet, stages II–V.
15–17. Development of fourth feet, stages III–V.
18–19. Development of fifth feet, stages IV–V, female.
20–21. Development of fifth feet, stages IV–V, male.

PLATE IV



Stage V

Body 1.24–1.40 mm. long, thorax of six segments, urosome of three segments in female and four in male, caudal rami asymmetrical, the right being longer and wider than the left (Plate III, Fig. 6). Through the successive stages there has been an increase in the length of both rami in proportion to their width, and the posterior outer marginal spine has assumed the character of the terminal bristles.

First antenna. Eighteen segments.

Second antenna. As in IV.

Mandible. As in IV.

First and second maxilla. As in IV.

Maxilliped (Plate III, Fig. 11). First basal with three long, strong bristles, one medium long bristle, and one short bristle. Inner branch as in IV.

First feet (Plate IV, Fig. 5). Coxopodite with one inner bristle. Basipodite with no bristle. Endopodite of three segments, the first with one inner bristle, the second with two inner bristles, and the third with six bristles. Exopodite of three segments, the first and second with one inner bristle, the third with two outer spines and five end and inner bristles.

Second feet (Plate IV, Fig. 10). Coxopodite with one inner bristle. Basipodite with no bristle. Endopodite of two segments, the first with three inner bristles, the second with eight bristles. Exopodite of three segments, the first and second with one outer spine and one inner bristle; the third with three outer spines, a terminal blade, and five inner bristles.

Third feet (Plate IV, Fig. 14). As second feet in V.

Fourth feet (Plate IV, Fig. 17). Coxopodite with one inner bristle. Basipodite with one outer bristle. Endopodite of two segments, the first with three inner bristles, the second with seven bristles. Exopodite of three segments, the first and second with one outer spine and one inner bristle; the third with three outer spines, a terminal blade, and five inner bristles.

Fifth feet female (Plate IV, Fig. 19). Symmetrical, similar to IV, but the end segment more elongate and bearing four small outer marginal spines.

Fifth feet male (Plate IV, Fig. 21). Asymmetrical, similar to IV, but with one strong and one weak outer marginal spine and two weak terminal spines.

Stage VI. Adult

Discussion

Tortanus discaudatus is a predacious species with specialized mouth parts for use in capturing and holding its prey, which consists largely

of copepod larvæ and other smaller copepods. This carnivorous habit is also present in the larval stages, where the recognizable food is made up of tintinnids, mainly *Tintinnopsis* and *Stenosomella,* two genera abundant in this locality. Nearly 50 per cent of the larvæ taken from the plankton contained loricæ of these ciliates during the season when they were numerous.

The long first antennæ, the large masticatory hooks on the second antennæ, the heavy hook on the gnathobase of the mandible, and the arrangement of the armature on the posterior end of the body serve to distinguish the nauplius larva of *Tortanus discaudatus* from that of any other described copepod larva. The closest resemblance appears to be to the larvæ of *Acartia longirèmis* and *Acartia bifilosa.* But this is a rather remote resemblance which is most noticeable in the shape and bristles of the mandibles and of the first antennæ. Other characteristics common to these species are the fusion of the first and second segments of the first antenna and the first and second segments of the exopodite of the second antenna. The furcal armature also recalls that of *Acartia.* Some resemblance was to be expected, for *Tortanus* and *Acartia* are remotely allied genera.

Table I gives the characters most useful in the identification of the nauplius stages of *Tortanus discaudatus.*

After the first nauplius stage the mandibles show but little change other than the strengthening of the spines and bristles and have therefore been omitted from the table. Size is perhaps the one most useful character, though the variations are sufficient to cause some confusion between two successive stages. The first, second, and sixth stages are comparatively easy to determine, while the third, fourth, and fifth occasion some difficulty.

In the fifth copepodid stage the animal has assumed practically all the adult structural peculiarities, and the changes that follow this stage relate mainly to sexual maturation and are mostly a matter of change in relative proportions. The moult from the fourth to the fifth copepodid stage is particularly significant in the development of the swimming feet. In the earlier stages each branch of each foot begins with a single segment and in the following moult the exopodite (and also the endopodite of the first feet) undergoes one division and remains thus until the change to the fifth stage, when each branch of all the feet (excepting the fifth pair) again undergoes one division, giving the total number of segments found in the adult. The fifth feet, especially in the male, are subject to considerable change on entering the sixth or adult stage, but it is to be noted that the right foot in the male did not become three-segmented, as described for the adult (*Corynura bum-*

TABLE I

Identification Table for Nauplius Stages of Tortanus discaudatus

		I	II	III	IV	V	VI
Length of body (in mm.)		0.12–0.14	0.185–0.205	0.23–0.26	0.30	0.32–0.34	0.36
First Antenna	Proximal segment	1 short bristle 1 long bristle	2 short bristles 1 long bristle	As in II	As III	As IV	As V
	Distal segment	3 terminal bristles	4 terminal bristles	As II but 2 dorsal and 1 ventral marginal bristle	As III but 4 dorsal and 1 ventral marginal bristle	As IV but 6 dorsal and 2 ventral marginal bristles	As V but 6 dorsal and 3 ventral marginal bristles
Second Antenna	Endopodite	2 terminal and 1 lateral bristle	3 terminal, 2 lateral bristles	3 terminal, 3 lateral bristles	3 long, 1 short terminal, 3 lateral bristles	As IV	As V
	Exopodite	6 bristles	7 bristles	7 bristles	7 bristles	8 bristles	8 bristles
Caudal spines		2 identical	2, the right longer, heavier	6 uneven length	6 uneven	6 uneven	6 uneven
First maxilla		0	0	Bud	Bud	Bud	Rudimentary
Second maxilla		0	0	0	Bud	Bud	Rudimentary
Maxilliped		0	0	0	0	Bud	Bud
First feet		0	0	0	0	0	Rudimentary
Second Feet		0	0	0	0	0	Rudimentary

pusii) by Wheeler (1899, p. 185, Fig. 21, *f*) but remains as figured by Thompson and Scott (1897, Plate 7, Fig. 2). The sexes are first distinguishable in the fourth stage by the structure of the fifth feet.

Following the moult to the sixth stage, a fifth segment is added to the urosome of the male, and in both sexes the urosome and caudal rami become suddenly much more asymmetrical and the lateral spine of the right caudal ramus becomes far more prominent. This asymmetry was indicated in earlier stages but remained latent and in this respect is similar to the characters accentuated by sexual maturation, with which it is doubtless closely associated. The asymmetry is most striking in actively spawning specimens and it has been noted that in the female the spermatophore is attached to the right lateral spine as well as to the genital segment. Asymmetry is first introduced with the second nauplius stage and persists through the following nauplius stages. Perfect symmetry again appears in the urosome and caudal rami of the first copepodid and persists also through the second and third copepodid stages. Following this there is a gradual small increase in asymmetry till the last sudden increase coming with the adult stage as mentioned

TABLE II

Identification Table for Copepodid Stages of Tortanus discaudatus

	I	II	III	IV	V
Length of body (in mm.)	0.55	0.74	0.96	1.11	1.24–1.4
First antenna	8 segments	11 or 12 segments	14 segments	16 segments	18 segments
Maxilliped, first basal	2 long bristles	2 long and 3 very short bristles	2 long and 3 short bristles	3 long and 2 short bristles	3 long, 1 medium, and 1 short bristle
Feet present	1st and 2nd (3rd as buds)	1st, 2nd and 3rd (4th as buds)	1st, 2nd, 3rd and 4th (5th as buds)	1st, 2nd, 3rd, 4th, and 5th	As in IV
Thorax	4 segments	5 segments	6 segments	6 segments	6 segments
Urosome	1 segment	2 segments	2 segments	3 segments	♀ 3 seg. ♂ 4 seg.
Caudal rami	Symmetrical	Symmetrical	Symmetrical	Right ramus slightly the longer	Right ramus the longer and broader

above. Plate III, Fig. 6, is for the female, but the male also shows a gradual change to asymmetry after the third copepodid stage and in the fifth stage the caudal rami are about as in the female but the lateral spine on the right caudal ramus is noticeably larger and there is a very slight indication of the pointed process found in the right distal corner of the first abdominal segment of the adult.

It will be noted that the appendages which change least during the successive moults of the copepodid stages are the second antennæ, the mandibles, and the first and second maxillæ. These are therefore of little use in determining the various stages.

Table II gives briefly some of the more salient features useful in identification of the copepodid stages.

Summary

1. Identification of the pelagic eggs of *Tortanus discaudatus* is established by hatching larvæ from eggs occurring in the plankton and also from eggs definitely known to have been spawned by the species in experimental cultures.

2. A description is given of the development of the specialized flotation case containing the egg.

3. The nauplius stages I–VI are described and figured.

4. The copepodid stages I–V are described and figures given.

5. The nauplius larva has a remote resemblance to the nauplius larvæ of *Acartia longiremis* and *Acartia bifilosa*.

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